VERTEBRATE PEST CONTROL AND MANAGEMENT MATERIALS: FOURTH SYMPOSIUM

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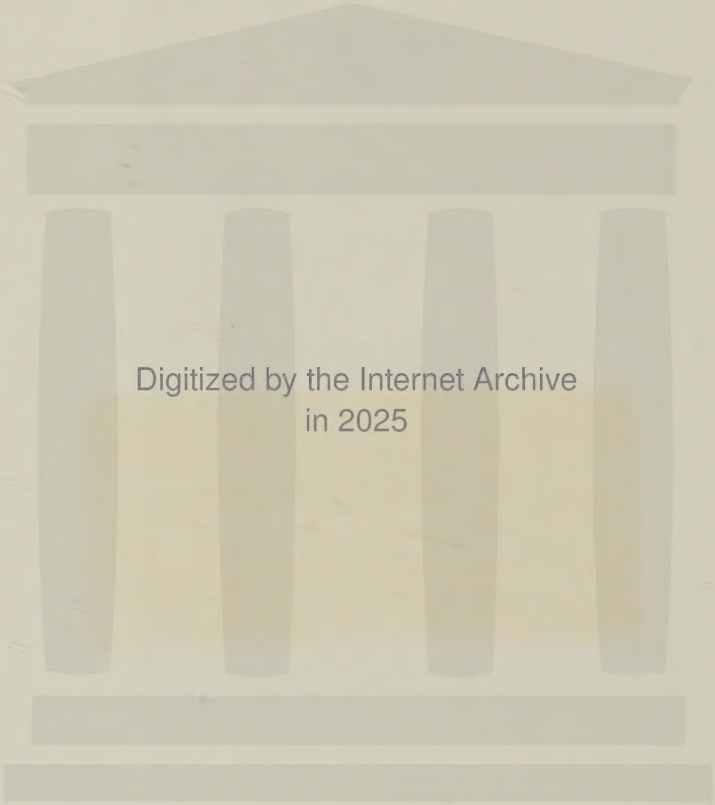
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VERTEBRATE PEST CONTROL AND MANAGEMENT MATERIALS: FOURTH SYMPOSIUM

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Foreword

The fourth symposium on Vertebrate Pest Control and Management Materials was held on 26 Feb. 1982 in Monterey, Calif. This symposium was the fourth in a series of symposia on test methods for vertebrate pest control and management materials. The sponsors of the symposium were ASTM Committee E-35 on Pesticides and its Subcommittee E35.17 on Vertebrate Control Agents, chaired by William B. Jackson, Bowling Green State University. The symposium chairman was Dale E. Kaukeinen, ICI Americas Inc., who also served as editor of this publication.

Related ASTM Publications

Vertebrate Pest Control and Management Materials (Third Symposium), STP 752 (1981), 04-752000-48

Vertebrate Pest Control and Management Materials (Second Symposium), STP 680 (1979), 04-680000-48

Test Methods for Vertebrate Pest Control and Management Materials (First Symposium), STP 625 (1977), 04-625000-48

Avian and Mammalian Wildlife Toxicology: Second Conference, STP 757 (1981), 04-757000-48

Avian and Mammalian Wildlife Toxicology (First Conference), STP 693 (1979), 04-693000-48

Aquatic Toxicology and Hazard Assessment: Sixth Symposium, STP 802 (1983), 04-802000-16

Aquatic Toxicology and Hazard Assessment (Fifth Symposium), STP 766 (1982), 04-766000-16

Aquatic Toxicology and Hazard Assessment (Fourth Symposium), STP 737 (1981), 04-737000-16

Aquatic Toxicology (Third Symposium), STP 707 (1980), 04-707000-16

Aquatic Toxicology (Second Symposium), STP 667 (1979), 04-667000-16

Aquatic Toxicology and Hazard Evaluation (First Symposium), STP 634 (1977), 04-634000-16

Ecological Assessments of Effluent Impacts on Communities of Indigenous Aquatic Organisms, STP 730 (1981), 04-730000-16

Aquatic Invertebrate Bioassays, STP 715 (1980), 04-715000-16

A Note of Appreciation to Reviewers

The quality of the papers that appear in this publication reflects not only the obvious efforts of the authors but also the unheralded, though essential, work of the reviewers. On behalf of ASTM we acknowledge with appreciation their dedication to high professional standards and their sacrifice of time and effort.

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Introduction

This volume, the result of a symposium held in Monterey, Calif., in February 1982, is the fourth in a series of symposia dealing with research techniques in the field of vertebrate pest control and management materials. These symposia have been sponsored jointly by ASTM Committee E-35 on Pesticides and the Vertebrate Pest Conference Committee. They have arisen largely because of the efforts of past editors and chairmen—namely, Rex Marsh, Bill Jackson, John Beck, Ed Schafer, and Charles Walker—and through the efforts of prior authors, reviewers, and ASTM editorial staff.

The purpose of ASTM Committee E-35 and its Subcommittee E35.17 on Vertebrate Control Agents is to develop standard definitions, classifications, appropriate test methods, and recommended practice statements for vertebrate management and control methods and materials. Standards and guidelines are produced by Subcommittee E35.17 in its regular meetings and in correspondence among members and nonmembers. While standards and guidelines may be addressed in these symposia, the allowable subject area and format are somewhat wider, providing a forum that includes control and management materials, papers on techniques, vertebrate pest damage assessment, review papers, and more general considerations or philosophies related to vertebrate control problems and research and to pesticide registration requirements.

What constitutes an ASTM paper for these symposia? The ASTM Special Technical Publications of the three previous symposia—*ASTM STP 625* (1977), *STP 680* (1979), and *STP 752* (1981)—contain a total of 80 papers giving the state of the art in this rather specialized area of interest. In examining these 80 titles, one could conclude that about 31% of previous symposia papers have dealt with pest rodents, 20% with birds, 14% with canines, and 11% with other categories such as ungulates, aquatic organisms, or general review or philosophical considerations. Laboratory or seminaturalistic simulated field test conditions were involved in 46% of the previous papers and 39% involved field conditions. About 32% of the previous papers dealt with toxicants and 30% with attractants, repellents, or antifertility agents. Damage assessment techniques were the subject of 11% of the previous papers.

Beginning with this symposium, the subject area has been somewhat modified because of the formation in 1980 of ASTM Committee E-47 on Biological Effects and Environmental Fate and its Subcommittees E47.01 on Aquatic Toxicology and E47.04 on Wildlife Toxicology. This new ASTM com-

mittee will provide an alternate "home" for the presentation of papers in certain areas that have previously been the concern of ASTM Committee E-35 and have been represented in its prior symposia.

Most of the previous papers at these vertebrate pest control and management materials symposia have had one or more of these main objectives: to develop methods and data for regulatory requirements pertaining to vertebrate control, to develop methods and data on vertebrate pest damage assessment (which is so needed in risk/benefit and cost/benefit considerations), and to optimize both materials and techniques for effective application to vertebrate pest problems.

The current volume continues to address these objectives, as well as to explore new areas. Some of the first basic pest rodent research is presented on the ancient, but newly popularized, glueboard trap, and the nature of rodent behavior in the penetration of packaging materials to obtain food is described. Further refinements in commensal rodenticide field evaluations are given, and new control approaches for these ubiquitous rodents are presented. Papers on field rodents involve newly verified abundance assessment techniques, techniques for control evaluations under various conditions, and work from countries with especially serious agricultural losses to rodents.

Papers in the pest bird area continue to evaluate damage and toxicant control, while increased attention is given to nonlethal management approaches such as distress calls and refined repellents. Canine predators, which may create nuisance or livestock depredation problems, are also represented by three papers concerned with lures, toxicants, and behavioral studies.

Two more general papers deal with both avian and mammalian concerns. One represents a significant milestone in allowing optimum and practical decisions to be made in vertebrate pest control through a systems approach that compares control options. Another provides a unique review of possibilities for utilizing existing basic behavioral knowledge of pest species to refine and optimize existing control and management approaches, as well as to suggest new approaches.

In 1977, Glen Sanderson of the Illinois Natural History Survey at Urbana, Ill., reviewed the proceedings of the first symposium, *ASTM STP 625*, in the December issue of the *NACTA Journal*. He noted that about 80% of the species and animal problem situations he had encountered were unrepresented, including bats, raccoons, tree squirrels, moles, muskrats, marmots, cottontail rabbits, and, particularly, bird problems in the farm and urban environment (starlings, pigeons, and house sparrows). Since 1977, some of these areas have been addressed in the two subsequent symposia, but there are still many subjects to be considered before this series can be in any way considered a comprehensive reference to the research in these areas.

It is heartening that new methods and materials continue to appear at this forum but somewhat disheartening that new animal problems continue to be

brought to general attention. Much work remains, and regardless of changes in political or social trends, most people directly involved in research in pest animal management and control will continue to find these symposia valuable references. I urge all who are involved in these areas to become participating members in ASTM and to consider sharing their experiences and expertise at future ASTM vertebrate pest control and management materials symposia.

Dale E. Kaukeinen

ICI Americas Inc., Biological Research Center, Goldsboro, N.C. 27530; symposium chairman and editor.

Terrell P. Salmon[1] *and Robert E. Lickliter*[2]

Comparisons Between Vertebrate Pest Control Materials: Essential Considerations

REFERENCE: Salmon, T. P. and Lickliter, R. E., **"Comparisons Between Vertebrate Pest Control Materials: Essential Considerations,"** *Vertebrate Pest Control and Management Materials: Fourth Symposium, ASTM STP 817,* D. E. Kaukeinen, Ed., American Society for Testing and Materials, Philadelphia, 1983, pp. 5–19.

ABSTRACT: When vertebrate pest control materials are evaluated and compared, biological, economic, and social considerations are all important. With regard to the biological and economic inputs, some of the more important points to be considered include the efficacy of materials, their usability and labor requirements, anticipated costs to users and consumers, associated hazards of each material, and the value of combining approaches when developing wildlife damage control programs. Through examples, we have demonstrated that the efficacy of any control material is a dynamic quality which varies according to season, species, locality, formulation, and use patterns, and that the interrelationship of these and other factors should be considered by decision-makers when attempting to compare the consequences of control material use, development, and regulation. We have also proposed a general outline for comparison of alternative vertebrate pest control materials.

KEY WORDS: vertebrate pest control, efficacy, pesticide evaluation, rodenticides, vertebrate pest control materials, animal damage control

Frequently, it is necessary to compare various vertebrate pest control materials such as toxicants, repellents, and traps. For example, comparisons are employed when selecting a specific material to solve an animal damage problem, when developing laws and regulations regarding the use of these products, or when deciding if new products should be developed and registered. Such comparisons generally involve an examination of the expected efficacy of the material in question, with some emphasis on the costs and labor required

[1]Extension wildlife specialist, Wildlife Extension, University of California, Davis, Calif. 95616.

[2]Staff research associate, Wildlife Extension, University of California, Davis, Calif. 95616.

for use. In many cases, this approach will lead to incomplete and inaccurate comparisons and may ultimately result in poor decisions.

Since comparisons and decisions regarding the selection, development, and regulation of vertebrate pest control materials are often made by people with limited experience or understanding in wildlife damage control, the purpose of this paper is to describe the kinds of information needed to accurately assess the anticipated properties of control materials and to present a general outline for their simultaneous analysis and comparison. In particular, we suggest that both biological and economic inputs be addressed in depth when comparing the consequences of using various control materials. With regard to biological and economic inputs, some of the more important points include (1) efficacy of materials, (2) their usability and labor requirements, (3) anticipated cost to users and consumers, (4) associated hazards of each material, and (5) the value of combining approaches when developing wildlife damage control programs.

Efficacy of Materials

Efficacy refers to a product's ability to produce the intended results. It is often used in a general sense to state the average control value of a particular material. Most efficacy figures are obtained from a limited number of quantitative laboratory and field studies and may not be representative of the various field conditions under which the material is used. Despite the fact that this type of information is used for registration purposes, it cannot be assumed that registered pesticides will be efficacious in all, or even most, situations. This has recently been demonstrated with pocket gopher (*Thomomys bottae*) control in alfalfa, where strychnine bait as presently used was not efficacious in two specific fields.[3] Since deviations from average product efficacy are common, examination of the various causes for these changes is important when specific materials are evaluated and compared. Important considerations for this type of evaluation include those discussed in the following sections.

Time or Season of Use

Seasonal factors such as animal behavior and bait acceptance drastically influence efficacy of materials. For example, strychnine bait is not usually considered effective in controlling Fisher ground squirrels (*Spermophilus beecheyi fisheri*). However, when this subspecies is pouching grain in the fall, strychnine bait can be a moderately effective control material [*1*]. Bait acceptance can also vary seasonally because of dietary preferences, and this is an important factor in determining the efficacy of many vertebrate pest control materials. For example, when California ground squirrels (*S. beecheyi*) emerge from hibernation, they feed primarily on forbs and grasses. At this time, they

[3]T. P. Salmon, unpublished research report, 1981.

do not normally consume the grain-type baits which are readily accepted in late spring and during the summer [2]. Similar preferences have been demonstrated for Belding's ground squirrels (*S. beldingi*) [3]. Climatic changes can also affect efficacy by influencing physical factors which affect the product. This occurs when controlling California ground squirrels by fumigation, which is effective only during periods when soil moisture is relatively high [1].

Locality

The location of the pest population can alter the efficacy of the material. For example, ground squirrels in one area may be controlled with zinc phosphide grain bait whereas another nearby population, for reasons not fully understood, is not controllable with this same material [4].

Annual Variation

The density of most vertebrate populations fluctuates from year to year. Although the exact causes of these fluctuations are unknown, weather, food availability, population density, and behavioral changes are believed important in regulating population numbers in specific areas [5]. Because of these year-to-year fluctuations, many control programs also vary in their ability to maintain vertebrate populations at desired levels. Even if the theoretical efficacy (for example, 80%) of a control material does not vary, the target population remaining after control may still be relatively large. As a hypothetical example, imagine a situation in which a product is 80% effective in controlling Norway rats (*Rattus norvegicus*). During years when rat populations are not expanding rapidly, the residual population (that is, 20%) after control may not cause serious damage. However, in years when weather conditions, food availability, and other factors are more favorable to rats, the residual population may expand rapidly and exceed the economic injury level. Apparently, this is what occurred in California in 1980–1981 when Norway rat populations increased beyond the economic injury level despite the use of the regular toxic baiting program.[4] In such cases, the efficacy of the total program must be improved if economic damage is to be prevented. This might be accomplished by using a combination of materials and methods, even though one material may, singly, be the most efficacious.

Variations Because of Multiple Use Over Time

With some rodenticides, multiple use over several years can change the material's efficacy as a result of bait or toxicant shyness, behavioral changes

[4]J. Williams, cooperative extension farm adviser, Sutter-Yuba Counties, Calif., personal communication, 1981.

in feeding methods or characteristics, or genetic resistance because of sublethal doses. For example, resistance to anticoagulants has been documented in Norway rats and house mice (*Mus musculus*) [6]. If cancellation or restriction of a particular pesticide results in more regular and repeated use of another pesticide, the efficacy of that material may decrease due to these factors.

Variations Because of Product Formulation and Ingredients

Active ingredients in vertebrate control pesticides are often the only factors considered in evaluation and comparison of materials, thus resulting in all formulations of a specific active ingredient being considered equivalent. In a recent report, the U.S. Environmental Protection Agency (EPA) stated that zinc phosphide was 60% efficacious in controlling California ground squirrels (*S. beecheyi douglasi*) [7]. This figure may be accurate as a general statement regarding the efficacy of zinc phosphide on this species, however, inert bait ingredients are often equal to and in some cases more important than active ingredients in determining product palatability and efficacy [3, 8, 9]. It is inappropriate, therefore, to generalize about the efficacy of a specific active ingredient without considering the efficacy of the various products available, as was demonstrated by Pank [10] during tests to control rats in sugar cane fields.

Species/Subspecies Variation

With many control materials, there are differences in efficacy between related species and subspecies. Assuming that the EPA's estimate of 60% efficacy for zinc phosphide on California ground squirrels is correct, this contrasts with the estimate of 85% efficacy for the Belding ground squirrel (*S. beldingi*) [7]. There are similar variations in efficacy between materials within a species. In one case, Marsh et al [11] found deer mice (*Peromyscus maniculatus*) more susceptible to the anticoagulant chlorophacinone than to diphacinone. Differences occur between subspecies as well. The subspecies of California ground squirrels (*S. beecheyi douglasi*) found in northern California is routinely controlled with strychnine-treated grain during late spring and early summer. During this same period, two other subspecies (*S. beecheyi fisheri* and *S. beecheyi beecheyi*) will not normally accept strychnine-treated grain [1]. Unfortunately, such differences are frequently overlooked by individuals unfamiliar with the species of concern.

Efficacy is not the sole determinant of the value or desirability of using a control material. This information must be coupled with the specific situation and objectives of the control program. Normally, higher efficacy equates with a better control material but lower efficacy may be acceptable in some cases. The usability of the material, its costs, and its hazards are some of the other factors to be considered.

Usability and Labor Requirements of Various Vertebrate Pest Control Materials

The ability of growers, pest control advisers and operators, homeowners, and others to use a vertebrate pest control material effectively will vary for many reasons, and this must be considered if adequate comparisons between products are to be made. Labor requirements are important factors in determining the usability of vertebrate pest control materials. Simple evaluation of labor cost differences for various control materials fails adequately to compare important differences between products. Some factors determining usability are discussed in the following sections.

Availability—The availability of most vertebrate pest control pesticides is determined by federal and state registrations and restrictions. Registration at one regulatory level does not ensure registration at the others, and this must be considered since products that are unavailable cannot be considered as alternatives. For example, thiram is federally registered as a ground squirrel repellent [*12*]; however, *no* ground squirrel repellents are registered in California [*13*].

Local Regulation—Irrespective of federal or state registrations, local regulations can also determine the availability or usability of materials. For example, in 1980 the Mendocino County, Calif., Agricultural Commissioner's office would not allow the use of strychnine-coated grain for pocket gopher control except in agricultural situations. As a result, golf courses and cemeteries were not able to use strychnine gopher bait despite both federal and state registrations which allowed this use. Although it would be difficult to determine specific local restrictions nationwide, this problem should be recognized and considered when suggesting label restrictions, cancellations, or alternatives to a vertebrate pest control material.

Source of Supply—Concomitant with registration of a product there must be an accessible supply. As an example, carbon bisulfide is recommended as a fumigant for controlling ground squirrels in California [*1*]; however, in most areas of the state, it is not available. This undoubtedly influenced the use of carbon bisulfide since, in 1979, only 2 of the 58 counties in the state issued permits for its use [*14*].

Marketing Restrictions—Marketing restrictions imposed by product manufacturers may also limit the product's usability. For example, the toxic perch is an alternative to strychnine bait for bird control; but, in California its availability is restricted by the manufacturer to one distributor.[5]

Special Requirements—Some products have special requirements for transportation, storage, or use which make them unavailable to certain users. Special application devices, sophisticated safety equipment, or specific environmental

[5]L. Martin, animal damage specialist, Blue Bird Enterprises, Fresno, Calif., personal communication, 1980.

conditions (such as moisture) can make certain materials impractical or unacceptable in some situations. This should be considered when alternatives to specific materials are discussed.

Special Training—The use of many control materials requires applicator training, and this can increase labor costs since an individual desiring to use the material must train himself or his employees, or both. It is unrealistic to expect individuals with relatively minor rodent problems to undergo extensive training in order to use a specific class of products. In these cases, the costs and efficacy of alternatives should be weighed against the anticipated time and cost of special training.

Certification—Many vertebrate pest control materials are classified by the EPA as restricted use materials and thus require the applicator to be certified. This increases both the cost and unavailability of labor. As a result, a nonrestricted material may be selected despite its lower efficacy.

Willingness to Use Vertebrate Pest Control Materials—Willingness to apply vertebrate pest control materials can vary according to the psychological and emotional feelings the user has about that material. Individuals often have personal biases about a material based on past experiences, rumors, and other sources of information. In such cases, reluctance to use the product could result in its less effective application or the use of more costly or less efficacious alternatives.

Time—When comparing the costs of various materials, the time required to apply each product is often a factor. In most situations, this practice fails to address the important question of availability of time. With many materials, the time (biological time) when it is effective is relatively brief, which causes its use to be concentrated during a specific period. A cogent example is fumigation for California ground squirrel control as an alternative to grain bait. It is relatively easy to determine the labor cost differential by comparing man-hours per hectare required for treatment with each product. However, this comparison can be quite misleading because it does not consider the feasibility or practicality of the proposed alternative. Fumigation is only effective when ground squirrels are active and when soil moisture is relatively high [*1*]. As a result, except in irrigated areas, fumigation is only conducted during a six- to eight-week period when natural soil moisture is high. The EPA documents supporting the strychnine rebuttable presumption against registration (RPAR) have made such a comparison and have resulted, in our opinion, in an erroneous comparison of two control materials [*15*]. In this case, fumigation was considered an alternative to strychnine for ground squirrel control in rangeland. According to the EPA, material and application costs for strychnine were $5.80 per hectare compared with $29.65 to $42.00 per hectare for fumigation. They estimated that 94 338 ha of rangeland in the United States is treated annually at a cost of about $543 000. If fumigation were used as the alternative to strychnine, the annual control costs would total about $2.1 mil-

lion, an increase of nearly $1.5 million. Apparently, they did not consider the magnitude of the fumigation program that would be required. While the time required to fumigate 1 ha of rangeland will vary, an application rate of 6.4 h/ha as used by the EPA would result in a labor commitment of 605 650 man-hours (75 706 man days) to fumigate U.S. rangeland currently treated by baits. If this labor requirement occurs during the short period of time when fumigation is effective, a very large labor pool would be required.

Another important consideration regarding time is the question of labor availability when the control material must be applied. If the need for applying the control material occurs simultaneously with other farm management practices, adequate amounts of labor may not be available. For this reason, growers sometimes select less efficacious vertebrate pest control methods or materials because they can be used when farm labor is available.[6]

Weather—Weather influences both the efficacy and usability of many vertebrate pest control materials. The biological time period when certain control materials or methods can be used may occur during poor weather, thereby preventing use. For example, ground squirrels (*S. beecheyi*) in almond orchards do not usually take grain baits well. There are short periods, however, when grain bait may be accepted, and it is during these times that a grain-baiting program would be conducted. However, grain baits are best used during dry weather conditions since cold, rainy, or wet weather tends to reduce rodent activity and bait acceptance, as well as increase bait degradation.

Costs of Various Vertebrate Pest Control Materials

The concerns discussed in the previous sections are important in comparing materials, and most of them will have a direct impact on the costs of a vertebrate damage control program. Although the actual material costs of various products are easily calculated, the application method also influences the cost of the control program. Certain materials need specialized application or storage equipment. For example, a large-scale ground squirrel fumigation program using carbon bisulfide would likely require development of specialized handling techniques and application equipment. Such additional costs must be factored into the evaluation of the product.

Economic Constraints—At times, even when the cost/benefit ratio to apply a material is favorable, the program is not adopted because of economic constraints. For example, if money is unavailable, growers may not invest in a control program, even if the expected benefit exceeds the costs. Additionally, the efficacy of a product or a control program is not guaranteed, so there is always economic risk with its use.

[6]T. M. Hardesty, manager, Reclamation District 2068, Dixon, Calif., personal communication, 1981.

Hazards Assessment for Various Control Materials

The hazards of using vertebrate pest control materials should be considered for the applicator, the potential nontarget species, and the environment. Since most materials have specific hazards associated with their use, these must be kept in perspective when comparing vertebrate pest control materials. As an example, a general comparison of strychnine bait and carbon bisulfide for ground squirrel control on ditch banks might reveal the following differences:

1. Applicator hazard—The applicator hazard using strychnine bait is minimal providing the applicator has been properly trained. Carbon bisulfide is extremely flammable and explosive and may be much more hazardous to use.
2. Nontarget species—The risks to most nontarget species from strychnine grain are potentially greater than with carbon bisulfide because nontarget exposure to carbon bisulfide is limited to the burrow system whereas strychnine grain is exposed above ground and is accessible to more species.
3. The environment—There is no evidence that strychnine accumulates in the food chain in significant quantities [*16*] or that it is phytotoxic. On the other hand, carbon bisulfide is highly toxic to plants and trees when their root zones interface with the fumigated burrow system [*1*]. The fire hazard from using carbon bisulfide is well known, and grass fires have been started by fumigating with this material. The effect of such fires on the environment and nontarget species could be significant if extensive habitat destruction results. Frequently, such changes make the habitat less desirable for nontarget wildlife as well [*17*].

Value of Combining Approaches When Developing Wildlife Damage Control Programs

Reliance on the "best method," that is, the most efficacious material, for a wildlife damage problem is not always the appropriate course. In fact, this approach may eventually lead to an inability to solve the problem. This occurs for many reasons, including those discussed in the following sections.

Resistance—Genetic resistance to anticoagulant rodenticides is well documented in commensal rodents [*6, 18*]. Resistance in field rodents has not yet been demonstrated; however, it is likely to occur if an active ingredient is used repeatedly over an extended period of time.

Bait/Toxicant Shyness—This phenomenon can occur with most acutely toxic baits. In general, bait shyness results from a sublethal exposure to the bait which produced toxic symptoms shortly after ingestion. The animal then associates the bait with the illness, and subsequent consumption of that bait will be reduced dramatically [*19*]. Frequent, repeated baiting with an acute toxicant that produces bait shyness can exacerbate the pest problem and lead to ineffective control with that bait. This has been demonstrated with California vole (*Microtus californicus*) control in sugar beets in San Joaquin County,

Calif., where zinc phosphide bait, after repeated application during the same season, became completely ineffective.[7] It is essential that alternatives be available in these situations, even if they are less efficacious.

Behavioral Shyness—Animals often learn to avoid certain materials. This has been demonstrated with traps, bait boxes, and, possibly, some baits [*20*] and is similar to the bait shyness phenomenon described earlier.

Timing—Wildlife populations and the damage they cause tend to fluctuate from year to year. Currently, there are no accurate prediction models to determine when and if a particular wildlife population will cause damage to specific crops. Since immediate action is often necessary when damage occurs, proper selection of the material and method used will be determined by each situation and is affected by many of the factors previously discussed. If several methods and materials are available, the most appropriate one for the specific situation should be selected.

Importance of Multiyear Control—Typically, the objective of a control program is to maintain the pest population below the economic injury level and thereby limit damage to an acceptable level. To achieve this with vertebrates, it is usually necessary to control residual populations in specific areas so they will not increase and invade fields or damage property. This can require the use of two or more materials or methods—perhaps one product to achieve the initial reduction and another product or method to control the remaining population.

The Location of Control Crop Versus Noncrop—Many vertebrate pests do not reside in cultivated crops and, therefore, must invade from adjacent areas [*21*]. In such cases, it is desirable to control them outside the crop area. This is particularly true if the pest species begins feeding on the crop, a condition which can lead to poor bait acceptance. Byers et al [*22*] reported this phenomenon with meadow voles (*Microtus pinetorum*) feeding on apples in an apple orchard. The issues of overwintering populations and invasion point sources must also be addressed when dealing with crop versus noncrop areas for control. For example, in the Tulelake area of California, the California vole is routinely controlled around ditch banks and in other noncrop areas. This has dramatically reduced vole damage to crops in that area.[8] Since this type of control program tends to reduce the population before it spreads over large areas, it will likely result in the use of less rodenticide in the area.

Discussion

A comprehensive comparison of vertebrate pest control materials must be made in terms of the predicted differences in their biological and economic effects. The interrelationships of these factors should be considered by decision-

[7]T. P. Salmon and W. P. Gorenzel, unpublished research report, 1980.

[8]K. Wright, agricultural commissioner, Modoc County, Calif., personal communication, 1982.

makers when deciding which control material is most appropriate for a specific situation. This is especially true when comparisons are used for regulatory purposes, since these decisions have far-reaching impacts which are not evident without thorough analysis of all possible alternatives. Manufacturers who make decisions regarding development or discontinuation of materials should understand how these decisions relate to other materials available for solving specific wildlife damage problems. Although all of the areas discussed previously cannot be addressed in every situation, significant limitations or weaknesses in comparative analysis should be identified in an attempt to minimize inaccurate or inappropriate decisions regarding vertebrate pest control material use, regulation, and development.

Adequate analysis of control materials must rest on a broad range of technical biological information and an equally broad, if less precise, judgment about economic impacts. Figure 1 serves to illustrate these primary concerns and demonstrates how a complete analysis can clarify judgments concerning comparisons of materials. While each component of the flow chart is complex and may require significant input, the resulting information should help to specify where considerations are inadequate or where they might have been overlooked. We also recognize that such a comparative process is subject to many of the comparison pitfalls we have discussed. However, arguing that adequate comparisons are impossible because of the complexities of this subject serves no purpose since comparisons must be conducted. If comparisons are conducted with the recognition that they are not absolute, we feel the process can have utility.

As illustrated in Fig. 1, use of the flow chart for the comparison of control materials should include (1) the estimated efficacy of each material to be compared, (2) the projected economic loss following proposed treatment, (3) the estimated labor and material costs of each material, and (4) the subjective evaluation of the potential hazards (that is, applicator hazard, nontarget species hazard, environmental hazard) and usability (that is, timing constraints, labor and permit requirement, product availability) of each control material. The relative ratings (low, medium, high) of each material's hazards and usability result in an overall "utility" rating for each material. When this is combined with the "projected economic benefit" for each material, a useful comparison of all materials analyzed can be made.

To illustrate the application of this flow chart, we will apply it to a specific pest problem (ground squirrels) in a specific crop (almonds). The object of our hypothetical analysis is to demonstrate the comparative process and not to serve as an actual comparison of control options. Information for the various control materials has been taken from two EPA reports [*7,15*]. While these data suffer from many of the problems we have discussed previously, the analysis will serve to demonstrate the flow chart. Additionally, we have estimated certain parameters for various control materials when necessary, again for illustration purposes. It is important to remember that use of the flow chart

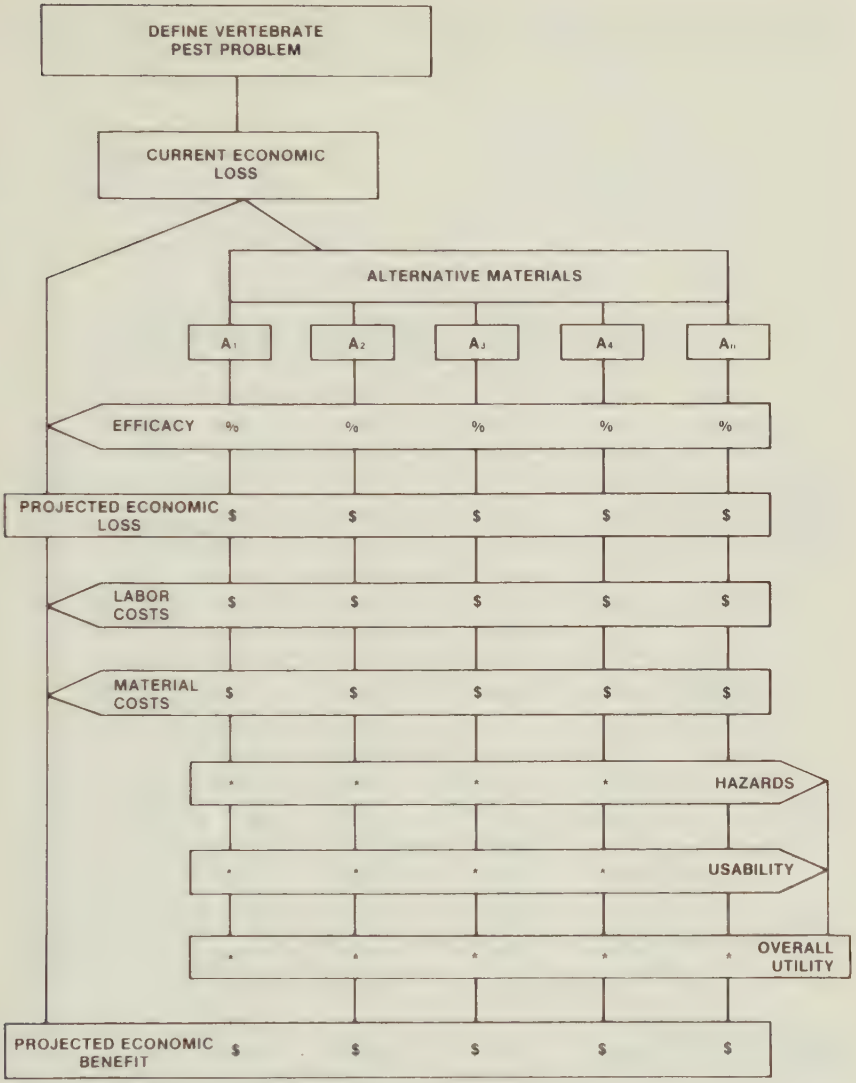

FIG. 1—*Flow chart for comparison of vertebrate pest control materials for solving specific wildlife damage problems.*

is relatively site and time specific. This is easily seen when the projected benefits of the various materials are compared for two seasons, spring (Fig. 2) and early summer (Fig. 3). Also, variations in efficacy, as previously discussed, will significantly influence actual comparisons, and the degree of influence must be evaluated through input from individuals with expertise in vertebrate pest control. Although it is possible to use such a comparison in a more general sense

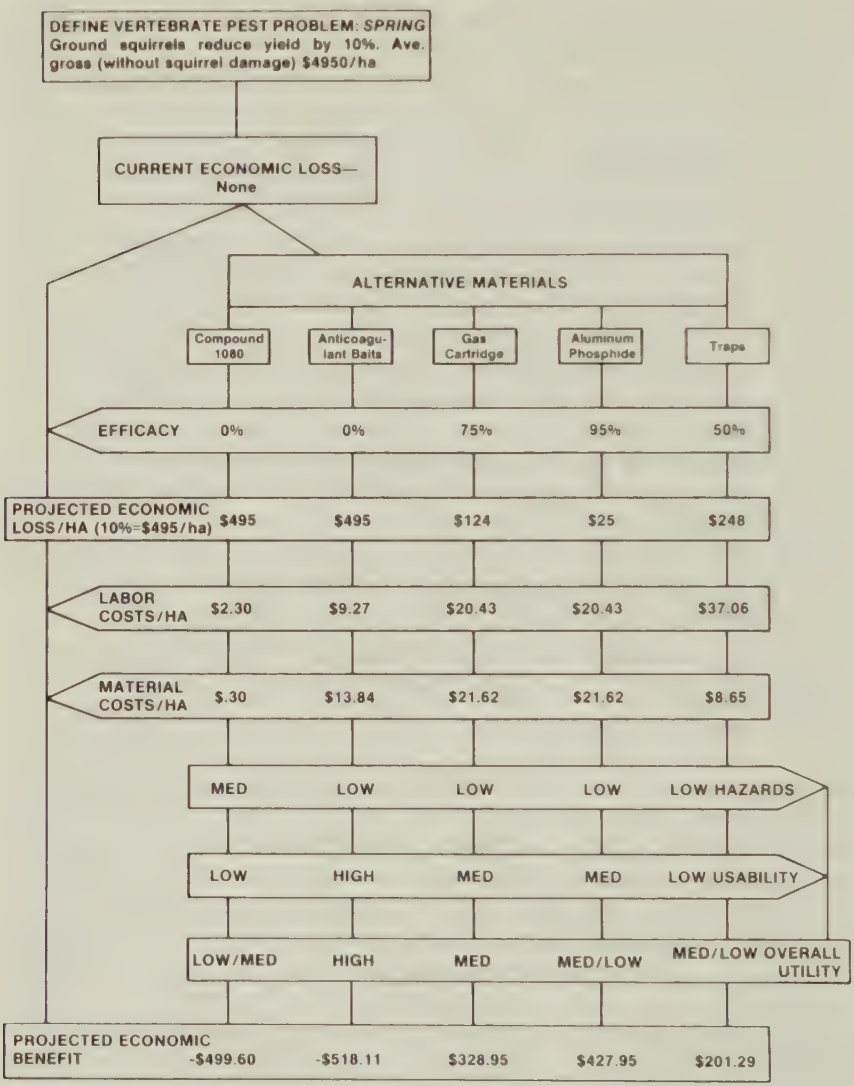
DEFINE VERTEBRATE PEST PROBLEM: SPRING
Ground squirrels reduce yield by 10%. Ave. gross (without squirrel damage) $4950/ha
CURRENT ECONOMIC LOSS—None
ALTERNATIVE MATERIALS
Compound 1080
Anticoagulant Baits
Gas Cartridge
Aluminum Phosphide
Traps
EFFICACY 0% 0% 75% 95% 50%
PROJECTED ECONOMIC LOSS/HA (10%=$495/ha) $495 $495 $124 $25 $248
LABOR COSTS/HA $2.30 $9.27 $20.43 $20.43 $37.06
MATERIAL COSTS/HA $.30 $13.84 $21.62 $21.62 $8.65
MED LOW LOW LOW LOW HAZARDS
LOW HIGH MED MED LOW USABILITY
LOW/MED HIGH MED MED/LOW MED/LOW OVERALL UTILITY
PROJECTED ECONOMIC BENEFIT -$499.60 -$518.11 $328.95 $427.95 $201.29

FIG. 2—*Hypothetical comparison of control materials for solving California ground squirrel damage in almond orchards during the spring months.*

(such as California ground squirrels and agriculture), severe data gaps on the economic impact of these animals and the variability of factors from one crop or location to another would make such comparisons difficult. For broad-scale decision-making, that is, federal pesticide registration, representative pests and sites could be used to overcome this problem.

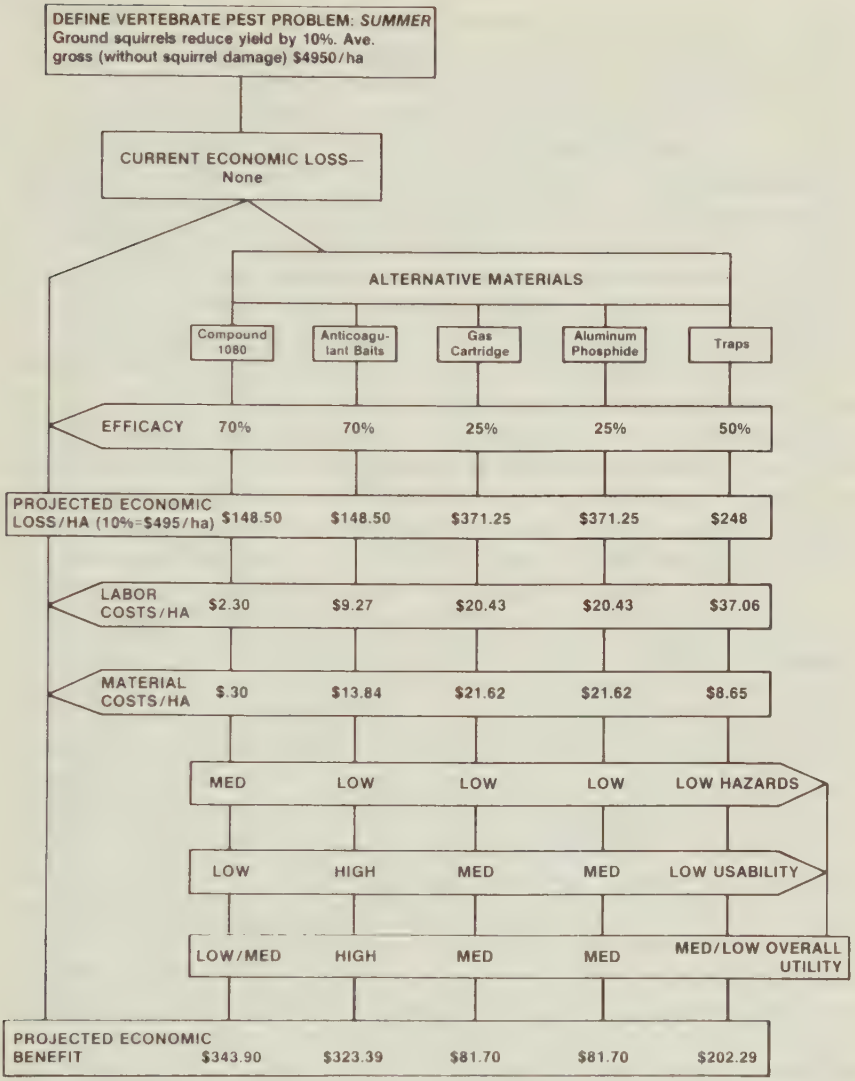
DEFINE VERTEBRATE PEST PROBLEM: SUMMER
Ground squirrels reduce yield by 10%. Ave. gross (without squirrel damage) $4950/ha
CURRENT ECONOMIC LOSS—None
ALTERNATIVE MATERIALS
Compound 1080
Anticoagulant Baits
Gas Cartridge
Aluminum Phosphide
Traps
EFFICACY 70% 70% 25% 25% 50%
PROJECTED ECONOMIC LOSS/HA (10%=$495/ha) $148.50 $148.50 $371.25 $371.25 $248
LABOR COSTS/HA $2.30 $9.27 $20.43 $20.43 $37.06
MATERIAL COSTS/HA $.30 $13.84 $21.62 $21.62 $8.65
MED LOW LOW LOW LOW HAZARDS
LOW HIGH MED MED LOW USABILITY
LOW/MED HIGH MED MED MED/LOW OVERALL UTILITY
PROJECTED ECONOMIC BENEFIT $343.90 $323.39 $81.70 $81.70 $202.29

FIG. 3—*Hypothetical comparison of control materials for solving California ground squirrel damage in almond orchards during the summer months.*

Conclusion

Comparison of the array of materials available for controlling specific wildlife damage problems is a difficult task. In fact, through our examples, it seems that thorough and complete comparisons would be virtually impossible. Nevertheless, comparisons must be made, and it seems appropriate to use

the most rigorous, practical approach, particularly with regard to regulatory decisions. No single measure or comparison can be adequate in making critical decisions regarding use of control materials. Rather, we have argued that biological and economic analysis provides a framework within which materials can be evaluated in a systematic manner and their positive and negative qualities presented in a useful form for decision-making. Careful analysis of alternative materials should point out unrealistic or questionable assumptions regarding their use, effectiveness, and cost/benefit ratios. The flow chart (Fig. 1) can be used for general guidance when comparisons are made, providing flexibility is incorporated to accommodate the specific aspects of each material that give it its unique value as a vertebrate pest control tool.

It is clear that comparisons of vertebrate pest control materials generate different values for different decision-makers. Agricultural producers will find comparisons most useful in the form of cost/benefit analyses, since appropriate comparisons can be used to maximize short-term or long-term profits, or both. They can also be used to assess the risk of wildlife damage problems and various potential solutions. Manufacturers can use comparisons to determine whether there is a need for additional products and, if so, what kind of products are most needed. For example, a comparison may show that a potential material with high-cost, high-labor requirements may be acceptable because available materials do not solve a specific problem. Regulatory agencies can use comparisons to clarify differences in alternatives and to determine the biological and economic impacts of regulatory decisions. Decisions based on such analyses are less likely to be misunderstood or opposed than are decisions based on less rigorous analysis and less objective criteria.

As we have shown, one vertebrate pest control material seldom replaces another without important trade-offs. Specialists in vertebrate pest control recognize the necessity for developing integrated management programs for solving wildlife damage problems. Such programs require the coordinated use of various control methods and materials, selecting the most appropriate materials for specific problems, seasons, and localities. Lack of control options due to lack of materials may seriously hamper the development of sound integrated management programs, a point that needs to be stressed, particularly with pesticide regulatory agencies.

Clearly, there is a need for decision-makers to identify and address the biological and economic factors associated with the use of vertebrate pest control materials. Of course, judgments and decisions regarding use, development, or regulation of control materials must be made by those responsible. The function of the comparative analysis process is not to replace these judgments; rather, it is to provide a method by which judgments and decisions can be improved and the likelihood of errors reduced.

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Russell F. Reidinger, Jr.,[1] *and J. Russell Mason*[2]

Exploitable Characteristics of Neophobia and Food Aversions for Improvements in Rodent and Bird Control

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ABSTRACT: Rodent and bird pests display a variety of behavioral defenses against dietary poisoning that could be exploited for improvement in control. Such defenses include neophobia, primary food aversion, and learned food aversion. Particularly with pest birds, social learning can also facilitate the transfer of learned aversions. With the rodents studied, taste appears critical for the defensive behaviors, whereas, with birds, visual or visual/taste cues appear more important.

With pest rodents, objective assessments of taste properties of rodenticides and baits could lead to improved formulations and methodology for objectively evaluating masking agents, microencapsulation, and prebaits. Assessment of the potential of a rodenticide as an unconditioned stimulus in food aversion learning, of the bait as a conditioned stimulus, and of other attributes such as generalization of learned aversions and conditioned stimulus/unconditioned stimulus intervals could lead to improved baits, baiting systems, and repellents. Weaknesses in the behaviors (for example, continuation of grooming in the presence of the defenses) might also be further exploited in rodent control.

With pest birds, Batesian mimicry probably offers advantages over food aversion learning as the conceptual basis of repellency. Use of visual cues with repellents such as methiocarb might improve aversion learning. Differential responses of the common chemical sense in birds and mammals to some compounds (such as dimethyl anthranilate) might also be used to advantage in some control situations.

KEY WORDS: bait, Batesian mimicry, bird control, common chemical sense, food aversion learning, grooming, neophobia, primary food aversion, rodent control, overshadowing, salience, smell, taste, vertebrate pest control, vision

Experts in vertebrate pest management have long known that successful applications of control compounds depend directly on an understanding of

[1]Wildlife biologist, U.S. Fish and Wildlife Service, assigned to the Monell Chemical Senses Center, Philadelphia, Pa. 19104, from the Denver Wildlife Research Center, Section of Supporting Sciences, Federal Center, Lakewood, Colo. 80225.

[2]Assistant member, Monell Chemical Senses Center, Philadelphia, Pa. 19104.

the animal's biology in the pest situation. Current recommendations for control are based on such aspects of the pest's biology as the distribution and identification of species, attributes of reproduction and development, social and feeding behaviors, physical abilities of the pests, and dynamics of pest populations [*1–3*]. More recently, efforts have focused on a different aspect of the animal's biology: its behavioral defenses against dietary poisoning. These defenses have probably received attention recently in part because there now exists a substantial literature on the attributes of the behaviors in rats. One of the defenses, food aversion learning, has recently been treated in reviews in which its potential applications to vertebrate pest management were discussed [*4,5*].

We are unaware of any published attempt to synthesize collectively the information available on the behaviors from the view of vertebrate pest management, and, indeed, such an attempt would be difficult even in a book-length presentation. In the absence of such a review, we have attempted here to present an overview on features of the behaviors that we believe are particularly important for further improvements in rodent and bird control. Further, we summarize some of the methods that are being followed to assess the potential uses of these concepts in vertebrate pest management and suggest some additional methods that we believe warrant consideration.

We emphasize here that the ideas are only presented as appearing reasonable from the view of our current knowledge of the pest's defenses against dietary poisoning and without regard to the myriad other considerations that need to be given to a potential control approach. The paper is, of necessity, theoretical, but wherever possible we include references to relevant literature which presents data substantiating our view. Because the sense of taste is central to many of the rodents' (and some birds') behavioral defenses, we define taste as referring to the qualities such as bitter, salty, sour, and sweet. For our purposes, flavors comprise both taste qualities and associated odors.

Behavioral Defenses Against Dietary Poisoning—Rodents

Three behavioral defenses against dietary poisoning are commonly described in the literature: neophobia (that is, avoidance of a new food or flavor), primary food aversion (that is, unlearned initial avoidance of a particular type of food, usually presumed due to bitter taste), and learned food aversion (that is, avoidance of a particular food acquired following its association with postingestional illness). In vertebrate pest management, food aversion learning is frequently referred to as bait shyness.

Neophobia

Our discussion will center on avoidance of novel edibles because of its obvious significance to vertebrate pest management, but neophobia appears to be

a more generalized phenomenon that can be directed to novel objects, locations, or odors as well as novel edibles in the animal's environment [*6–9*]. In his recent review, Domjan [*10*] described such neophobia as a suppression in consumption of novel edibles and summarized the factors that are known to attenuate or enhance this type of neophobia. As one would expect, neophobia for edibles is attenuated by repeated exposures to a tastant, with attenuation increasing gradually over several hours following presentation of the stimuli. The attenuation can be retained for several weeks and, if the food stimuli are presented as fluids (as is commonly done in laboratory studies), attenuation generalizes along a solution-concentration gradient. Other authors imply a dynamic characteristic for this type of neophobia. Presumably, neophobia would be strong and reliable during periods when the diet of the animal meets its nutritional needs. However, the animal would become non-neophobic or even neophilic in situations in which the diet fails [*11*]. Such a phenomenon might explain the observations of neophilia in some field situations, for example, the tendency of ricefield rats (*Rattus rattus mindanensis*) to consume even novel baits such as oats or corn during the vegetative stages of rice growth (if one presumes that rice stems are a nutritionally deficient diet for the rats) [*12*].

Domjan [*10*] also described illness-mediated neophobia and food aversion learning–mediated neophobia, two instances in which neophobia for edibles can be enhanced. Illness-mediated neophobia occurs when a rodent becomes sick in the absence of a readily associable cue (such as salient taste) and results in avoidance of new tastes for the period of sickness. Food aversion learning–mediated neophobia is similar to the illness-mediated form, but the rodent remains neophobic for the duration of the learned aversion.

Although we recognize the importance of neophobia in development and use of bait formulations, experimentally the concept has received only limited attention by scientists working on vertebrate pest management.

Primary Food Aversion

Laboratory rats reject solutions of quinine sulfate at 0.000008 *M*, and quinine hydrochloride at 0.0003 *M* or less [*13*]. The ecological rationale for such rejection is that the bitter taste signals a poisonous material to a rodent, particularly since many of the naturally occurring toxins (such as alkaloids and glycosides) are presumed to taste bitter to rodents [*14*]. Three points require additional comment. One is that not all rodents appear equally sensitive to bitter tastes. Notable examples are wild and domestic guinea pigs [*15*], the former of which reject quinine hydrochloride only at concentrations of 0.002 *M* or more [*13*]. Such a range of sensitivity can probably be expected among pest rodents as well.

A second point is that perception of bitterness, at least in humans, appears relatively more complex than perception of other taste qualities [*16*]. What

we commonly refer to as bitter taste may, in fact, involve several different taste receptor systems [*17*].

The third point is that the actual taste properties of most control materials to pest rodents are unknown. Only recently has the experimental methodology that would allow such empirical assessments become available [*18*]. Without such methodology, it must be assumed that references in the literature to the taste properties of rodenticides and baits (for example, Ref *3*, p. 22) are based on either human taste experiences [*19*], or the observed unpalatability of the material (presumed to be due to bitter taste). Since the limited information available indicates that a material does not necessarily taste the same to humans as it does to rodents [*20,21*], inferences from human taste experience to that of rodents must be regarded with caution.

Food Aversion Learning

Food aversion learning has received by far the greatest research attention among those defensive behaviors of importance in rodent control. A summary of its general attributes in relation to animal damage control was presented by Dorrance and Gilbert [*4*] and others, but we will describe additional applications of the attributes. Food aversion learning occurs experimentally when a rodent is presented with a distinctive-tasting food [conditioned stimulus (CS)] followed by a postingestional illness [unconditioned stimulus (US)]. In most laboratory studies [*22*], the "food" is actually presented as a solution to be drunk by the rodent. There is now little doubt that the resultant aversion involves learning, although the interval of time between presentation of the CS and the US, during which learning can occur, may be much greater than for many other forms of learning studied in the laboratory and although food aversions occur after only a single pairing of food with postingestional illness. It is of interest that the US can be any of a variety of illness-inducing agents (such as irradiation, drugs, or toxins), although some compounds such as lithium chloride (LiCl) appear to be the most reliable in producing learned aversions.

Another attribute of this form of learning for rodents is the important role of taste. Although it has been suggested that all that is required for formation of a food aversion is a taste followed by a postingestional illness [*23*], some tastants are more likely to be associated with sickness (that is, they are more salient) than others. In general, novel tastants appear more salient than familiar ones (at least when the rodents have an adequate diet, as is true in most laboratory experiments that have been conducted) [*24*]. In situations in which the rodent is given confounded CSs (that is, more than one tastant before the US), the more salient tastant overshadows the other, and the rodent subsequently avoids that taste [*25,26*]. It is also of interest that, with the possible exception of sodium deficiency wherein the rodent exhibits a "specific hunger" for sodium [*27*], the rodent seems unaware of the actual

cause of its US-induced illness. The rodent seems aware only of being sick and more or less automatically associates its sickness with a salient taste.

A final attribute of food aversion learning, important for its potential applications to vertebrate pest management, is that once a rodent has formed an aversion to a specific taste, the aversion generalizes to other compounds having similar tastes. Theoretically, the strength of avoidance is proportional to the similarity of the taste of the compound to the taste of the CS [*24*] and to the intensity of the US.

Other Defensive Behaviors

Although neophobia, primary food aversion, and learned food aversion constitute major mechanisms by which individual rodents can avoid or learn to avoid dietary poisoning, some social mechanisms also exist through which such avoidance can be transferred within the pest population. Galef [*28*] and colleagues have demonstrated a variety of social behaviors that probably allow transfer of a learned food preference (and consequently avoidance) from adult to young. Their data demonstrate that the flavor of a mother's milk, for example, can have important influences on the dietary selection of a weanling rat [*29–32*]. Weanling pups are probably also influenced by the odors and physical presence of adults at a potential feeding location [*28*]. More recently, Pettijohn [*33*] has shown that learning of an aversion can be socially facilitated in the Mongolian gerbil (*Meriones unguiculatus*); that is, the gerbil can learn a food aversion by observing another gerbil suffer postingestional illness.

Importance of Taste in the Defensive Behaviors

We emphasize that, with the possible exception of some of the social defenses (and nontaste forms of neophobia), the sense of taste appears to play the critical role in the rodent's defenses against dietary poisoning. As summarized by Garcia et al [*11*], inherited preferences, past sensory and postingestional experience with a food, and nutritional needs (in turn based on factors such as food availability and physiological condition) converge to adjust the relative palatability of a potential food. The index of such adjustments are principally the qualities of taste, however, and taste determines whether an item is accepted or rejected.

The Rodent as a Food Expert

Given the arsenal of behavioral defenses just described, it is apparent that one must assume the rodent pest is a food expert within its own environment [*11*]. One can also speculate on the steps through which a bait must pass if a lethal amount is to be consumed by the rodent pest. Assuming that the ro-

dent has an adequate diet, the pest would likely be neophobic in its first encounter with the bait. If the rodent's diet is inadequate, such a step may not occur. Given time for familiarity, the rodent would then sample a small amount of the bait. If the bait has an inherently bad taste (presumably bitter), the rodent is likely to avoid it (primary food aversion). If the bait does not taste inherently bad, the rodent would presumably ingest some of the bait and await postingestional effects. How much is ingested at this feeding would appear to depend heavily on past experiences that the rodent has had with similar-tasting materials. Assuming that the rodent survives this ingestion, it would have learned to avoid the bait subsequently (that is, learned a food aversion), the strength of subsequent avoidance being dependent on such factors (described previously) as the nature of the illness, the amount consumed, the salience of the taste of the bait, the interval of time between ingestion (the taste serving as the CS) and its effects (the toxin within the bait serving as the US), and extinction (that is, complete attenuation) of the learned aversion.

Potential Relationships of Rodent's Behavioral Defenses to Rodent Control: Hypotheses, Applications, and Methodologies

The exciting consequences of these descriptions is that they can serve as the conceptual bases for potential improvements in our current approaches to rodent control. The concepts can be used to develop control-related, testable hypotheses and, in many instances, the methodologies that would be required to test the ideas. In this section, we summarize such ideas according to their applications to the development of rodenticides and bait formulations, the development of rodent repellents, and alternative control approaches.

Potential Applications to the Development of Rodenticides and Baits

Here we suggest several ways that knowledge of the attributes of the rodent's defenses might be used to increase the palatability of a bait and to reduce the opportunities for bait shyness.

Description of Taste Qualities of Baits and Rodenticides—As already discussed, taste appears to play the key role in determining whether or not a rodent will accept a bait, but the taste qualities of rodenticides and bait formulations to rodents are essentially unknown. A starting point would be research to assess the taste properties of such formulations. Interestingly, one method that would provide this information is based on an attribute of food aversion, generalization from the taste of the CS to other similar tastes. Initial findings from such an approach are presented elsewhere [*18*]. Information on taste qualities of baits as well as methodology for obtaining such information could have important uses in rodent control. For example, the determination of taste qualities of these compounds would allow an objective evaluation of the idea

that bitterness is the major taste quality involved with primary food aversion. Such methodology would also allow comparisons of bait formulation and rodenticides to determine which are least likely to elicit primary food aversion with a specific pest species or population. Further, the methodology would allow empirical and fundamental assessments of such approaches as microencapsulation and of bait additives such as masking agents (for example, see Ref *18*) and bait enhancers.

Robbins [*34*] recently demonstrated, with *Peromyscus maniculatus* and *Mus musculus*, that a prebait was more effective if its flavor closely resembled that of the actual bait [that is, sodium chloride (NaCl) was added to the prebait to mimic the taste of the subsequently used toxicant, LiCl]. Robbins [*34*] suggested that the effect was due to a decreased ability of the rodents to learn a food aversion rather than the more commonly held view that prebaits serve to reduce neophobia and to establish a momentum to consume. He therefore suggested that a prebait should be designed to resemble as closely as possible the flavor of the bait (even if this reduced the palatability of the prebait) rather than the more common practice of making a prebait as palatable as possible (and not necessarily resembling the flavor of the bait). As suggested by Robbins [*34*], identification of the taste qualities of rodenticides would allow the formulation of nontoxic mixtures of tastants that would mimic the rodenticides' tastes. The addition of these mixtures might improve the effectiveness of some baits.

Finally, we believe that descriptions of the taste qualities of baits and rodenticides can be viewed as the first step toward the systematic formulation of baits. Conceivably, if one knew the taste preferences of the rodent in the pest situation, one might be able to formulate a bait whose taste qualities were closely matched to the taste preferences of the pest.

The Toxicant as an Unconditioned Stimulus—We believe that a rodenticide's potential as a US in food aversion learning also warrants systematic evaluation. To our knowledge, Nachman and Hartley [*35*] have presented the only published information comparing rodenticides as conditioned stimuli. Their results indicated that the rodenticides tested varied in their potential as USs. Presumably, a rodenticide whose mode of toxicity is readily associated with postingetional illness is also likely to cause bait shyness. Thus, objective evaluation of the compound's potential as a US might be one criterion for determining the compound's potential for problems associated with bait shyness. Nachman and Hartley [*35*] described the basic method for this type of evaluation, although Robbins [*36*] has recently suggested an improved protocol, specifically for the evaluation of rodenticides as unconditioned stimuli, that involves intubation rather than intraperitoneal injection of the rodenticide-US (to improve ecological validity), and two-choice rather than one-choice drinking tests (to improve sensitivity of measurements). As described in detail by Robbins [*36*], the relative consumption of the tastant ver-

sus plain water in the two-choice drinking tests [*37*] as well as the number of days to extinction would probably indicate the compound's potential as a US. By standardizing such parameters as the CS, the concentration of the CS, and the CS/US interstimulus interval, it would likely be possible to compare the potential of various rodenticides as USs for a given pest species. An additional advantage of the procedure suggested by Robbins [*36*] is that it would allow separate measurements of the degree of bait shyness due to food aversion learning and that due to food aversion learning-mediated neophobia.

The Bait as a Conditioned Stimulus—Systematic assessment of a bait formulation's potential as a CS in food aversion learning might also be exploited for improvements in bait formulation. Presumably, tastes of some bait formulations are more salient than those of others for a given pest species and control situation. The more salient the taste of the bait, the more likely the bait is to cause bait shyness. An intriguing working hypothesis is that an attractive bait, particularly one that incorporates tastes that are novel to the pest, is also likely to be a salient bait [*38*]. To our knowledge, the hypothesis has not been explicitly tested, although workers have demonstrated that familiar foods are sometimes preferred baits [*39*]. For example, Bullard and Shumake [*40*] have shown that rice volatiles (that is, familiar odors) enhance the palatability of food for the ricefield rat, *R. rattus mindanensis*. If substantiated, the hypothesis brings into question the generally accepted idea that a bait formulation, particularly one in which an acute rodenticide is used, should be as attractive as possible to the rodent pest. In our opinion, the desirability of such a practice has not been demonstrated in many control situations. The idea that salience may be an important but overlooked criterion for the development of baits at least warrants additional study. To test the relative potentials of two baits to be associated with postingestional illness, one could present them as confounded CSs, followed by injection of a US such as LiCl, and determine in subsequent preference tests which bait overshadows the other. Kalat and Rozin [*25*] and Lindsey and Best [*26*] have already described such methods. Presumably, this approach would serve as a criterion for determining which bait is least likely to cause bait shyness.

The CS/US Interval—Assessment of CS/US intervals for rodenticides might also warrant consideration in development of bait formulations. Presumably, the strength of aversion (and, ultimately the ability of the animal to form an aversion) is inversely related to the CS/US interval [*23*]. In laboratory studies involving USs other than rodenticides, an animal is usually capable of forming a taste aversion if the CS/US interval is 5 to 6 h or less, although such associations have been reported with an interval as great as 24 h [*41*]. In bait shyness, of course, the bait serves as both the CS and the US, so the question of CS/US interval really becomes a question of delayed toxicosis (that is, the interval between the ingestion of the bait and onset of sickness). Again, we are unaware of literature that has specifically assessed this factor

for rodenticides. However, onset of symptoms has been generally recognized as an important consideration in the assessment of potential rodenticides. For example, Savarie [*42*] recently noted that "it is rather surprising that of the thousands of organophosphorous chemicals that have been synthesized only one, phosacetim (Gophacide), has ever been evaluated as a practical rodenticide. The unusually fast onset of action of organophosphates probably precludes the ingestion of enough bait to cause mortality; however, the poisoning symptoms associated with phosacetim apparently develop slowly, enabling the rodents to consume lethal doses." Others [*43*] have also recently described the importance of time of onset of symptoms in their studies of scilliroside with bandicoots (*Bandicota bengalensis*).

An interesting speculation, related to CS/US interval, is that the relative lack of bait shyness attributed to the traditional anticoagulant rodenticides, such as warfarin, might be due in part to an unusually long delay between ingestion and onset of toxic symptoms. In support of this notion is the common knowledge that anticoagulant poisons require repeated feedings and several days to take effect. Experimentally, one might be able to assess the time delay between ingestion of a rodenticide and onset of symptoms by presenting the rodent with a salient taste and by varying the intervals of time before intubation with the rodenticide. By measuring the avoidance of the tastant in subsequent preference tests, one could estimate the time course for onset of symptoms that are associated with sublethal doses of the poison and perhaps establish dosage levels (or select compounds or methods of microencapsulation, for examples) that are least likely to cause bait shyness.

Generalization of Learned Food Aversions—Finally, we suggest that another attribute of food aversion learning, generalization of learned aversion to other foods (or baits), could have important applications for the development and use of baits. For example, in situations in which more than one rodenticide is being used, it seems likely that use of baits having similar tastes would encourage the generalization of a learned aversion from one bait to the other. In the tropical developing world, a recommended procedure for control of rodents in rice fields, for example, is sometimes the use of an acute rodenticide such as zinc phosphide to reduce the population, then sustained use of a chronic rodenticide such as warfarin to maintain the population at a low level [*44*]. In at least some situations (we suspect many), the farmer uses the same bait (sometimes a highly attractive bait), changing only the rodenticide, an approach likely to encourage transfer of bait shyness from the acute to the chronic rodenticide by surviving pests. Generalization of learned aversions from one bait to another would appear empirically testable in the laboratory. For example, one could allow the rodent to taste a sublethal amount of the bait (or a prebait whose taste properties mimic the taste of the bait, followed by injection of a US), and then test for subsequent avoidance of the alternative baits. Other factors being equal, the least avoided bait in the subsequent tests would be the best match for sequential baiting with the CS-bait.

Potential Applications to the Development of Rodent Repellents

Two general approaches to rodent repellency have been attempted. With one, the items to be protected are treated directly with an aversion-causing formulation. This was the approach used by Lindsey et al [*45*], for example, to protect fir seeds from deer mice (*P. maniculatus*) with mestranol, and by others [*46.47*] with endrin. With the other approach, used sometimes to protect crops, a bait that tastes similar to the crop is used to afford "safety" to the crop. Presumably, after ingesting the bait, the rodent learns to avoid the taste of the bait and generalizes the aversion to the taste of the crop. Tevis [*48*], for example, coated conifer seeds with sodium fluoroacetate in a modestly successful attempt to protect untreated seeds from *Peromyscus*. A problem, observed by Tevis [*48*] and demonstrated by Robbins [*34*] in the laboratory, is that the rodents, on repeated exposures, learn to discriminate between the taste of the untreated crop (or seeds) and the taste of the bait (or treated seeds) and thereafter eat the crop and avoid the bait. Assuming that the food aversion is not absolute (that is, that the rodent will again sample the bait), learning of such fine taste discriminations could be predicted from other studies on food aversion learning in rodents [*49*]. One possibility, analogous to our earlier discussion on prebaits, would be to treat the crop with a nontoxic formulation that mimics the taste of the toxin. There are, however, other promising approaches.

Direct Application of the Repellent to the Crop—If the crop is treated directly, as exemplified by the work of Lindsey et al [*45*], one could speculate that the formulation should (*a*) taste novel to the pest species involved (to encourage at least temporary avoidance due to neophobia), (*b*) induce primary food aversion (that is, presumably taste "bad" to the pest), (*c*) have a highly salient taste (to encourage food aversion learning), and (*d*) contain a toxin that (*e*) serves as a strong US (also to encourage food aversion learning) but that is (*f*) nonlethal (if repellency rather than mortality is desired). The approaches and methods suggested in the preceding section on baits would appear to be also adaptable for testing potential repellent formulations to determine whether they meet these criteria (except *f*). With the goal of repellency, of course, one would try to optimize avoidance rather than to minimize it.

New Approach to Repellency—As a new approach to repellency, we suggest that consideration be given to using a bait that is placed near the crop (to encourage sampling of the bait and the crop in a confounded manner) and whose taste is relatively less salient than that of the crop (to encourage overshadowing and misassociation of the learned aversion with the taste of the crop rather than with that of the bait). Thus, the approach would depend on a potential weakness in food aversion learning: the presumed unawareness by the rodent of the cause of its sickness (US) and more or less automatic association of the sickness with a contiguous, highly salient taste (CS). To make the taste of the crop relatively more salient than that of the bait, one would likely have to (*a*) increase the salience of the crop or (*b*) decrease the salience of the

bait. As an example of the former approach, one might treat the crop with a novel and highly salient but nontoxic tastant. Shumake[3] and others have begun some studies related to this idea. Or, alternatively, one might examine the salience of tastes of other pesticides that are routinely sprayed on the crop to determine if they might also be exploited in this manner. An advantage of the latter approach (*b*) is that one might not have to treat the crop at all. A potential problem, however, is that a nonsalient bait might also be unattractive. One might have to use an alternative to feeding or drinking, such as grooming, as a means of delivering the bait to the rodent pest. Reidinger et al [*38*], using laboratory strains of *Rattus norvegicus*, have recently demonstrated that use of grooming in this manner is at least theoretically feasible.

Development of Alternative Control Approaches

Heretofore we have concentrated on the attributes of the defensive behaviors as they might be exploited for improvements in bait formulations and rodent repellency. We now suggest that the collective natures of the defensive systems might also constitute a weakness that is currently underexploited in rodent control. There is evidence, both anecdotal and experimental, that the behavioral defenses of the rodent are particularly well suited for protecting the rodent during its major ingestive activities, feeding and drinking (also the most common means of delivering rodenticides to the rodent), but that the defenses may be less well adapted for protecting the rodent during other activities in which ingestion is a minor, but essential, component. Such activities include grooming, nest building, and gnawing. Grooming has been used in the control of vampire bats [*50*], and nest building has been considered for use in rodent control [*51*]. Ingestion by grooming is considered essential for the effectiveness of tracking powders and dusts [*2,3*], and Marsh [*52*, p. 61] has stated that rodents tend to ingest aversive materials while grooming that they would not ingest while drinking or feeding. Pank[4] and others have also conducted studies showing that wild rodents will ingest lethal quantities of acute rodenticides while grooming. To our knowledge, however, there has not been a systematic assessment of the relationships of grooming behavior to the animals' defenses against dietary poisoning.

We have begun such an assessment using laboratory strains of *R. norvegicus*, with the objectives of describing the characteristics of food aversion in the grooming context as well as the effects of food aversion on the grooming behavior of rodents. Based on the results from these studies, it appears that laboratory rats perceived the taste of a material while grooming it from the fur and gathered information as well on the concentrations and qualities

[3]S. Shumake, Denver Wildlife Research Center, Federal Center, Lakewood, Colo. 80225, personal communication, July 1979.

[4]L. Pank, Wildlife Damage Research Station, Hilo, Hawaii 96720, personal communication, October 1975.

of the tastants involved [*38*]. Further, the rats learned aversions to a taste perceived in the grooming context and generalized the aversion from the grooming to the drinking context. Similar results were obtained with laboratory strains of mice, *M. musculus*,[5] but results from studies with pine voles, *Microtus pinetorum*, were less clear.[6]

Nonetheless, rats that had formed strong aversions to the taste of saccharin continued to groom, with some modifications of the components of grooming, after a material containing saccharin was applied to the fur.[7] Further, in studies conducted with pine voles, the authors[8] found that grooming an aversive tastant from the fur was associated with increased levels of corticosteroids in the blood. The increase in blood levels of the steroids occurred in a rodent when grooming an aversive tastant from its own fur but not when grooming a cagemate. Since corticosteroids are believed to stimulate a strongly stereotyped form of grooming in rodents [*53*], the authors[8] postulated that the pine voles had become caught in a deviation-accelerating, positive feedback loop [*54*] in which grooming resulted in the perception of the aversive tastant, perception of the tastant resulted in more grooming, and so on.

If this postulate is confirmed, and occurs in other pest species, grooming in the presence of an aversive tastant may be a more robust phenomenon than was previously thought (judging from its limited use in rodent control) and might have more uses in rodent control than previously considered. For example, it might be possible to use, by means of grooming, some of the chemosterilants, emetics, and acute rodenticides that have been judged impractical because of problems with poor acceptance.

Behavioral Defenses Against Dietary Poisoning—Birds

Many of the defenses that protect rodents from dietary poisoning also protect birds. However, because pest birds are often granivorous and social,[9] their feeding behavior is frequently guided by visual rather than taste stimuli [*55*] and strongly influenced by the social context in which stimuli are presented [*56,57*].

Attributes of Neophobia, Primary Food Aversions, and Food Aversion Learning

Neophobia—Little information is available about neophobia in wild birds, although insects apparently use neophobic responses of birds to their advan-

[5]C. Stewart and R. F. Reidinger, Jr., unpublished manuscript.
[6]L. Geyer, C. Kornet, and R. F. Reidinger, Jr., unpublished manuscript.
[7]R. F. Reidinger, Jr., unpublished data.
[8]J. R. Mason and R. F. Reidinger, Jr., unpublished manuscript.
[9]J. R. Mason and R. F. Reidinger, Jr., unpublished manuscript.

tage [*58*]. In laboratory and controlled outdoor settings, neophobia for some visual stimuli can exert strong control over behavior [*59,60*]. This is especially true for young birds. For example, Rabinowitch [*61*] raised two groups of chickens (*Gallus gallus*) on different monotonous diets (wheat or milo seeds) and then switched each group to the other group's diet. Half of the birds in each group did not sample the novel food and starved. Likewise, Hogan [*62*] has reported that naive chicks do not attack meal worms when they are first presented, but instead exhibit fear and escape behavior. Herring gulls (*Larus argentatus*) and ring-billed gulls (*L. delwarensis*) behave similarly [*63*]. In most cases, however, neophobia is transitory [*56*]. Because individual birds also exhibit neophilia [*64*], we suggest neophobia, *per se*, is an unpromising approach to bird damage control, although it may enhance the efficacy of other strategies.

Primary Food Aversions—Like neophobia, primary food aversions in birds are strongly influenced by visual cues. Colors such as red or orange are especially likely to elicit avoidance, whereas colors such as green or blue are less likely to do so [*58,65*]. Tastes also elicit primary aversions, but such aversions are relatively weak when compared with aversions elicited by visual cues and vary widely among species, strains, and individuals [*66*]. Even substances such as capsaicin or citric acid, which are rejected by most mammals [*67*] and suggested as bird control agents [*68*], are not aversive to some birds (such as parrots and redwings) and may be preferred [*66*].[10] Moreover, the aversiveness of some tastants is enigmatic when birds are compared with mammals. For example, birds uniformly reject food or water containing dimethyl anthranilate, a commonly used artificial grape flavoring [*69*]. Finally, primary taste aversions in birds are often context dependent. Birds, more than mammals, are sensitive to testing procedures (for example, the number of alternatives offered), lighting, temperature, humidity, and other environmental variables. For such reasons, we suspect that conspicuous colors (which elicit relatively uniform avoidance) may be more effective than offensive tastes (which do not elicit uniform responding) in eliciting primary food aversions in birds. Because primary aversions to conspicuous colors also diminish [*70*] unless combined with other forms of conditioning, however, we suspect that food aversion learning may be more useful in programs of bird damage control.

Food Aversion Learning—As in neophobia and primary food aversion, visual cues are important for food aversion learning by granivorous birds (that is, the birds learn to avoid visual cues paired with foods whose ingestion has been followed by sickness) [*71–73*]. As in food aversion learning by rats [*74*], the strength of the aversion learning is a function of the intensity of the visual cues [*73*], some visual cues overshadow others [*75,76*], aversions generalize to

[10]Chili peppers are commonly recommended as food for parrots and are offered for sale by commercial dealers. See, for example, the price list of bird foods offered by W. G. Scarlett & Co., Baltimore, Md. 21202. Also, we have observed that some redwings show unlearned preferences for capsaicin in two-choice drinking tests.

different intensities of the visual cue paired with sickness [*73*],[11] and associations can be formed over long delays between cue presentation and sickness [*77, 78*].

It is especially interesting that birds can learn food preferences and aversions by observing conspecifics, and without necessarily ingesting food themselves [*56, 79–83*]. Such observational learning is guided by the visual characteristics of the food, although, in some cases, tactile and gustatory stimuli as well as the long-term consequences of ingestion may become important [*84*] Regarding preferences, chaffinches (*Fringilla coelebs*) are more likely to commence feeding and sample new foods when exposed to conspecifics doing so [*85*]. Wagtails (*Motacilla alba*) [*86*] and Tennessee warblers (*Vermivora peregrina*) [*87*] behave in a similar fashion, and starlings (*Sturnus vulgaris*) may increase feeding efficiency while minimizing energy expenditure through social feeding [*88*]. Such feeding is discriminative, and individuals tend to choose foods that other birds in the flock are choosing [*89, 90*]. Such discriminative feeding might contribute to the selective crop damage produced by gregarious birds such as redwings [*53*]. Regarding aversions, redwings [*57*] and other species [*58*] learn to avoid visual stimuli associated with foods whose ingestion has sickened conspecifics. Not surprisingly, observational learning in an aversive context can extend beyond foods *per se*. It appears that male starlings can learn to recognize and avoid conspecifics present during an aversive feeding experience.[12]

Batesian Mimicry

Food aversion learning is widely regarded as the theoretical basis of bird repellency [*91, 92*]. Although that basis is sound, we feel that the more inclusive concept of Batesian mimicry may be more useful [*5*]. In Batesian mimicry, palatable organisms (mimics) are avoided by predators because of their resemblance to (mimicry of) unpalatable organisms (models) that the predators avoid [*58*]. The fundamental mechanism by which predators learn about models and mimics is food aversion learning [*93*], but the importance of other behavioral phenomena (such as observational learning) is also stressed [*58*]. In the mimicry model of bird repellency, the "predator" would be the bird, the "model" would be the portion of the crop treated with repellent, and the "mimic" would be the untreated portion of the crop. We suspect that birds experiencing the aversive consequences of eating model crop or observing the consequences on conspecifics [*94–96*] would avoid mimic crops as well.

Applications and Methodologies

The two most commonly used bird toxicants are 4-aminopyridine and methiocarb. One (4-aminopyridine) is applied in a bait [*97*], whereas the

[11] J. R. Mason and R. F. Reidinger, Jr., unpublished manuscript.
[12] J. R. Mason and R. F. Reidinger, Jr., unpublished manuscript.

other (methiocarb) is applied directly to the crop [*98*]. We suggest that problems associated with 4-aminopyridine are roughly analogous to problems associated with the use of baits to induce crop aversions in rodents. That is, it would not be surprising if, on some occasions, birds observing the lethal consequences of toxicant on conspecifics develop aversions for the bait rather than for the crop, since the bait is conspicuous (for example, a different color from the crop or other background against which it is applied). Methods that reduce the conspicuousness of 4-aminopyridine baits might enhance the possibility of observational learning of aversions for the crop, although such methods might also increase the difficulty of enticing birds to the baits, given that birds are visual feeders.

Unlike 4-aminopyridine, the repellent methiocarb has considerable potential from the perspective of Batesian mimicry. As currently applied, it is colorless and presumably nonconspicuous, which perhaps explains why, in the absence of distinctive color cues, methiocarb is sometimes a poor US [*92*] We suspect that the ineffectiveness of methiocarb with some crops [*99*] is not due to the crop's failure to overshadow cues presented by the repellent. Rather, the birds may be familiar with the cues presented by the crop. Such familiarity with conditioned stimuli weakens learned food aversions in the laboratory [*100*], and field evidence indicates that methiocarb applications are less effective if depredating birds have been damaging a planting for several days before application [*101*]. The addition of conspicuous color cues (that is, bright colors or simple patterns) should reduce if not eliminate familiarity effects.

As in Batesian mimicry, with which birds learn to avoid conspicuously colored models, depredating birds should rapidly learn to avoid a distinctively colored crop. Such learned avoidance *per se* could increase the efficacy of methiocarb applications. Moreover, we speculate that the birds would generalize the learned avoidance to a harmless, but distinctively colored, crop. Evidence consistent with this notion is provided by a study in which perches coated with glue effectively repelled Philippine weavers (*Lonchura* spp.) from rice fields [*102*]. Although the glue itself remained effective (that is, it caught birds who subsequently exhibited distress) for only three days, birds avoided the fields having the perches for at least an additional five days. Apparently, the visual presence of the perches in the fields was an adequate cue for expression of observationally learned avoidance in these species.

Distinctive colors need not be applied directly to either model or mimic crops [*56,57*] and, at least in the laboratory, models and mimics need not be the same variety of food as long as the color cues are the same [*71*]. Even a poor resemblance between the model and mimic affords the mimic some protection [*58,103*]. Issues such as the proportions of models to mimics and the distribution of models among mimics have not been addressed, but testable hypotheses can be derived from published work [*70,104,105*]. Provided that the birds are sensitive to the toxicant and not given extensive experience with

the models and their mimics, the mimics will receive some protection in a population containing up to 70% mimics and only 30% models. While birds can learn to make fine discriminations between models and mimics [*106*], such discrimination seems to occur only after extensive experience and, apparently, only when the models are weakly repellent [*107*]. We suggest that by creating essentially perfect mimics (for example, untreated corn) of adequately repellent models (for example, methiocarb-treated corn), and by using mimicry only with crops having a limited period of vulnerability to bird damage, the efficacy of methiocarb could be enhanced. Even if a high number of models were required to protect mimics, reductions in the cost of methiocarb through the application of inexpensive dyes could produce a net economic benefit [*108*].

Another Behavioral Defense Against Poisoning: The Common Chemical Sense in Birds

Recent evidence suggests that birds have sensitive nasochemoreceptive capabilities. Wallraff [*109*] has shown that pigeons can use olfactory cues to home over distances as great as 50 km, and evidence from our laboratory suggests that birds have acute trigeminal, or common chemical sense, abilities and that such abilities can be used to mediate both primary and learned food aversions. Recently, we trained starlings to avoid volatiles from 2-phenylethanol, a floral scent that mainly activates the olfactory system in mammals. Bilateral transsection of the olfactory nerves had no effect on responses, but plugging of the internal and external nares with denture adhesive and cyanoacrylate glue reversibly abolished the aversion. Because birds have no vomeronasal system, such results implicate the trigeminal system as mediating the aversion learning.[13] The possibility exists that other compounds that are effective trigeminal irritants for birds (but not for mammals) could be found. Such compounds could serve as useful components of strategies aimed at controlling damage through repellency.

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Birds

Robert H. Schmidt[1] *and Ron J. Johnson*[2]

Bird Dispersal Recordings: An Overview

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ABSTRACT: Bird damage control often involves dispersing birds from areas where they cause problems. Dispersal techniques have been used at airfields, rural and urban bird roosts, livestock facilities, fruit orchards, grain fields, and other locations. Certain avian vocalizations have evolved as alarm or distress calls, and these calls can be exploited as a means of dispersing birds. The behavioral response to such calls, however, varies. Certain species may disperse with the appropriate call, whereas others show little or no reaction. The efficacy of this technique is not well documented at present, but its potential for development as a management tool seems great.

Visual warning signals may increase the effectiveness of bird dispersal recordings by decreasing the habituation rate, increasing realism, or decreasing the fright threshold of the birds causing problems, or by a combination of these factors. Habitat manipulation, which reduces the attractiveness of an area to birds, complements dispersal efforts. It appears that a combination of management techniques is the most effective strategy. In addition, pretreatment evaluation of the problem and situation plus records of field results are helpful parts of a dispersal effort.

Evolutionary, theoretical, and applied aspects of bird communication are discussed as they relate to bird dispersal, the repellency of recorded sounds, habituation rate, and effects of regional dialects. A review of the vocalization and hearing ranges of birds is included; this may help define the frequency and type of sounds most likely to disperse birds. The characteristics of recording and broadcasting equipment are detailed in relation to component selection, and suggestions are made for effective use. A section on field application of bird dispersal recordings provides guidelines for duration and spacing of playbacks and recommends using an integrated approach. Continued refinement of bird dispersal recordings and associated techniques can increase considerably our effectiveness in solving bird damage problems.

KEY WORDS: vertebrate pest control, birds, dispersal recordings, bird communication, alarm calls, distress calls, auditory repellents, habituation, regional dialects, recording equipment, broadcasting equipment, playback considerations, biological control, airport management

[1]Research technologist, Department of Forestry, Fisheries and Wildlife, University of Nebraska, Lincoln, Neb. 68583-0819; presently graduate student in Ecology, Wildlife and Fisheries Biology, University of California, Davis, Calif. 95616.

[2]Extension wildlife specialist, Department of Forestry, Fisheries and Wildlife, University of Nebraska, Lincoln, Neb. 68583-0819.

Twelve years after Thomas A. Edison first displayed his tinfoil phonograph to the American public in 1877, a German, Ludwid Koch, made what is believed to have been the first recording of a bird's voice [*1*]. In 1900, the first recording of a bird in the wild was made, and by 1946 bird sounds were being recorded on magnetic tape [*2*].

As more recordings and better equipment became available, biologists around the world began to use these recordings to learn more about avian taxonomy, ecology, and behavior. In 1953, Hubert Frings and Joseph Jumber found that starlings (*Sturnus vulgaris*) emitted a "piercing shriek" when handled. They reported that "this 'distress call' caused other starlings to fly out of the barns immediately, even in the dark, and they did not return, even after some months" (Ref *3*, p. 318). It occurred to them that a recorded distress call could be used as a repellent, and they successfully used those recordings to clear starling roosts in two towns in central Pennsylvania. Frings continued his work [*4–7*], and various European researchers also began recording bird sounds and testing their effectiveness in dispersing birds [*8–10*]. They were generally successful and found that a wide variety of birds responded to distress or alarm sounds, or both. From these pioneering beginnings, the use of bird dispersal recordings has now become a common technique used in many integrated bird damage control programs.

For convenience and clarity, we will define bird dispersal recordings as electronic reproductions of sounds, usually on magnetic tape, which are intended to disperse birds when broadcast. These sounds can have either natural or artificial origin, but currently most of the recordings available are of natural sounds (Schmidt and Johnson, unpublished data).

Bird Damage Problems and Dispersal Recordings

Damage Problems

During the course of history, man has hunted, tamed, and even worshiped birds. In today's modern world, we find ourselves more and more in conflict with some species of birds for the use of the same food and space resources [*11*]. With the world human population projected to be 6 billion to 7 billion by the year 2000 [*12*], the demand for food and fiber production can only increase. In addition, land use changes have favored certain wild bird species. Some of these, such as starlings and blackbirds, are economically important and are generally on the increase across much of North America [*13*]. Kozicky and McCabe [*14*] listed some areas of conflict that exist between humans and birds: (1) consumption or destruction of foodstuffs, (2) economic losses to nonfoodstuffs, (3) hazards to aircraft, (4) transmission of disease to man and domestic animals, and (5) negative effects on man's comfort, aesthetics, and sporting values. It is very unlikely that these conflicts will be decreasing in the foreseeable future. Bird dispersal recordings have potential applications in all of these areas.

Because of advances in technology, some problems that did not exist 50 years ago are severe today. Bird hazards to aircraft is an example. In separate essays on the economic impact of birds in relation to man, Henderson [*15*] stated that gulls are "practically harmless" and Du Pay [*16*] agreed that they were "seldom harmful to man." But with man's increasing presence in the skies and the advent of turbine engines on aircraft, gulls now account for 40% of the world's reported bird/aircraft strikes [*17*]. Actually, the first reported instance of a fatal bird/aircraft strike occurred in 1912, when Cal Rodgers, the first man to fly across the United States, died after a gull became entangled in the control wires of his aircraft, causing it to crash [*18*]. Although birds may cause human death indirectly by consumption or destruction of foodstuffs or through transmission of disease, they are a direct and obvious danger when they interfere with the normal operation of aircraft. In 1960, an Electra in Boston collided with a flock of starlings soon after takeoff, resulting in a crash landing and 62 fatalities [*19*]. Harrison [*20*] noted that from 1966 to 1976, the U.S. Air Force experienced 3851 bird strikes which resulted in damage or loss of aircraft, with 9 fatalities and 18 aircraft lost. The estimated monetary loss was at least $81.1 million, not including the man-hours required for repair. Clearly, birds can cause significant problems whenever there is competition for the same airspace [*21–24*].

Approaches to Bird Dispersal

Numerous techniques have been employed for dispersing nuisance, depredating, or dangerous birds. These include shouting, arm-flapping, banging sticks against metal pans, scarecrows, bird decoys, raptor kites or balloons, falconry, shooting, propane exploders, pyrotechnics, synthetic alarm devices, recorded bird sounds, rock music, microwave radiation, ultrasonic sounds, chemical repellents and frightening agents, changes in cultural practices, and habitat manipulation [*23,25–27*]. Although using a single technique has been effective in some situations, generally the best approach to bird damage problems is to use several complementary techniques together [*14,23,24,26*]. No one technique works best for all locations, weather conditions, and species.

Zabadal and Hothem [*28*] reviewed some of the disadvantages of many bird dispersal techniques. These include (1) the small range of influence for many devices, (2) the ability of birds to habituate to certain audio and visual stimuli, (3) the fact that many techniques are labor intensive, and (4) the annoyance factor caused by the disturbance to the human environment.

Bird dispersal recordings have a number of advantages over other dispersal techniques. When used properly, they can be a valuable and useful technique for dealing with problem birds around airports [*23*], agricultural concerns [*29–31*], urban and rural structures [*32,33*], and in other problem situations

[*7,34*]. Generally, the initial cost of the system is the only major expenditure, and this cost can be prorated over the length of the control program. The equipment necessary is readily available and can be operated manually or automatically. Because the recordings are usually distress or alarm sounds, the biological or natural basis of the sounds may decrease the chance of habituation before the operation is completed. With increasing public concern over the treatment of animals, nonlethal control methods, such as bird dispersal recordings, are likely to receive more public acceptance than lethal techniques [*35,36*]. However, the limited range of bird dispersal recordings may limit their cost-effectiveness in protecting very large areas.

Mention should be made of dispersal recordings other than alarm and distress calls. Work on developing a superstimulus through the analysis of alarm and distress sounds [*9,37*] has not proven fruitful. Synthetic sounds may lack biological significance, thus habituation may be more rapid [*38,39*]. Music has received some support as a dispersal tool [*28,40,41*], but it may not elicit a response too different from that of other sounds that are nonbiological in origin.

Methods

Primarily four methods were used to review and evaluate bird dispersal recordings and to locate current sources. First, we reviewed the literature. Second, we requested information through correspondence. A questionnaire was sent to 285 individuals or agencies, and letters of inquiry were mailed to 42 potential commercial sources of bird dispersal recordings. The questionnaire asked for information including sources of recordings, resource people to contact, techniques to use, and comments or opinions. It was mailed to Cooperative Extension Service personnel in all 50 states, all U.S. Fish and Wildlife Service-Division of Animal Damage Control state offices, all participants in the Bird Hazards to Aircraft Training Seminar and Workshop held 8–9 Sept. 1976 at Clemson University, and other selected biologists from the United States, Canada, and Great Britain. Of the 285 questionnaires mailed, 131 (46%) were completed and returned; the actual number of respondents was probably higher, because many agencies coordinated their responses through one individual, thus eliminating duplication. Third, two poster displays were used to elicit information about bird dispersal recordings and their potential uses. One was presented at the 42nd Midwest Fish and Wildlife Conference, 7–10 Dec. 1980, in St. Paul, Minn.; the other was presented at the 1981 Midwest Regional Animal Behavior Meetings held 27–29 March 1981, in Ames, Iowa. Fourth, we traveled to visit in person with 21 professionals experienced with bird dispersal recordings and to view tape library facilities.

Characteristics of Bird Sounds

Bird Communication

Sound is only one segment of a bird's communication repertoire. Other communication systems include visual, tactile, and possibly other signaling devices [*42*]. One reason communication systems exist is because they are energy efficient; for example, it takes less energy for a male bird to attract a female by singing than by flying out, locating, and bringing a female back.

Acoustical signals have the advantage of being invisible and easily produced, and they do not require special lighting conditions or media. Acoustical signals travel fast and do not linger or clutter the sensory environment because they dissipate rapidly. One disadvantage of acoustical signals is that they can be exploited by predators [*43*].

The vocalizations of birds have been intensively studied for many years; birds are a popular behavioral subject and have an extensive "vocabulary" of sounds. In comparison, other vertebrate animals generally do not have as varied an acoustical repertoire [*44*]. As an example of the potential complexity of avian vocalizations, the red-winged blackbird (*Agelaius phoeniceus*) has 20 adult vocalizations [*45*]. Within this repertoire various vocalizations have specific meanings and functions. Marler [*46*] found that the vocabulary of the European chaffinch (*Fringilla coelebs*) included a flight call, a social call, an injury call, an aggressive call, three distinct and separate escape (alarm) calls, three courtship calls, song, and subsong. Clearly, the acoustical communication system of birds (even excluding nonvocal sounds) can be quite complicated.

How Birds Produce Sound

Some understanding of the mechanisms and limits of avian vocalizations is helpful in working with them effectively. Birds have a unique sound-producing organ called the syrinx, located at the junction of the bronchi and the trachea [*47*]. Greenwalt [*48*] reviewed many of the hypotheses on the mechanisms and operation of bird vocalizations. Briefly, as air is forced out of the lung/air-sac system that forms a bird's respiratory apparatus, membranes located in the syrinx (the internal and external tympaniform membranes) vibrate and produce sound. Muscles connected to the syrinx control the tension of the membranes and thus affect the characteristics of the fundamental frequency [*47,49*]. Some birds are capable of producing sounds separately from each bronchial passage [*47,48*]. Birds with a more complex syrinx structure generally have a greater frequency range but not necessarily a more complex song [*48*].

Vocalization Ranges

There are limits to the frequency ranges of vocal sounds produced by birds. Greenwalt [*48*] reported that for the birds in his study, the frequency ranges of songs free of harmonic content varied from a low of 80 to 90 Hz for the spruce grouse (*Dendragapus canadensis*) to a high of 10 700 Hz for the brown-headed cowbird (*Molothrus ater*). Brand [*50*] found the average frequency for 59 different species of birds to be 4280 Hz, with the highest song noted belonging to the blackpoll warbler (*Dendroica striata*), which had a high note of 10 225 Hz. For comparison, the lowest C note on a piano has a frequency of 32.7 Hz and the highest C is 4186 Hz [*49*].

Following are the frequency ranges reported by Brand [*50*] of some species that are considered a hazard to aircraft.

American crow (*Corvus branchyrhynchos*)	1450 to 1650 Hz
American robin (*Turdus migratorius*)	2200 to 3300 Hz
Cedar waxwing (*Bombycilla cedrorum*)	7675 to 8950 Hz
Starling (*Sturnus vulgaris*)	1100 to 8225 Hz
Red-winged blackbird (*A. phoeniceus*)	1450 to 4375 Hz

This limited sample shows that species exhibit different frequency ranges. In his study of North American bird sounds, Greenwalt [*48*] reported the greatest range in the song of the brown-headed cowbird, embracing an interval of 700 to 10 700 Hz. Harmonics associated with the fundamental frequency can occur at even higher frequencies than reported for harmonic-free songs. Their role in avian communication is not well understood.

Hearing Ranges

In the past, reports have suggested that birds' hearing ranges are similar to man's [*48*], which is approximately 16 to 20 000 Hz [*51*]. Bremond [*52*] reported that most birds hear between 100 and 20 000 Hz. However, recent studies have shown that pigeons and probably many other birds sense frequencies as low as 0.05 Hz [*53*], well below the range of human hearing. It seems unlikely that birds hear sounds which are above the range of human hearing [*7,21,34,49,54,55*].

Thorpe [*51*] speculated that birds do not necessarily hear tones of the same range that they produce. Pumphrey [*56*] reported that "it is most unlikely ... that 10 kilocycles represents the upper limit for small birds since recognition of a specific song or call seems to require perception of at least the first few harmonics as well as the fundamental of the highest tone in the song" (p. 141). He later revised his position [*57*], stating that he "doubts whether there is any useful sensitivity above 10 kilocycles per second in birds other

than owls and perhaps parrots" (p. 82), based on the physical characteristics of the avian ear. Bremond [*52*] further clarified this issue by pointing out that "perception and emission of high frequencies would be only of slight value as their range in air is limited" (p. 735).

The upper frequency of songs or calls can be increased by the generation of harmonics associated with the fundamental or first harmonic. Greenwalt [*48*] found that for a given species there is a threshold frequency below which harmonics occur and above which a harmonic-free whistled phrase is generally given. This threshold ranged from 500 to 4000 Hz. He noted that Passeriformes generally had harmonic-free songs, whereas in nonpasserines, phrases with substantial harmonic content were relatively common.

As harmonics increase in frequency, some begin to exceed the upper limit of the avian ear (greater than 20 000 Hz). Higher frequencies attenuate rapidly and become inaudible at correspondingly shorter distances from the sender. Thus, in a song with a fundamental frequency of 4000 Hz one or two harmonics may be audible to other birds, but it is unlikely that any above this would be heard because even the second and third would probably be faint [*58*]. Harmonics are limited by the upper limit of the hearing range and by the rapid loss of energy associated with high frequencies in general. This does not mean that they have no importance. For many bird species harmonics above the first predominate in at least a portion of the song [*48*] and thus play a role in species recognition. Based on the hearing ranges of birds, it is unlikely that high-frequency sounds would be effective for bird dispersal work. Additionally, we found no evidence that ultrasonic devices are effective for this purpose [*25,54,55*].

Time discrimination (temporal resolving power) refers to the ability of an organism to distinguish between separate successive sounds which can be heard as distinct within a given time interval [*51*]. Pumphrey [*57*] reported that time discrimination in birds was at least ten times better than in humans. Greenwalt [*48*] believes that this figure is low and argues that time discrimination is 50 to 100 times better in birds than in humans. He concludes that "there is then the strong presumption that birds hear *as such* the rapid modulations so characteristic of their songs, and that the information content even in relatively simple songs must be enormous" (p. 142).

In summary, bird vocalizations free of harmonics generally fall between 100 and 11 000 Hz, or within the hearing range of most birds. Harmonics increase the frequency range of the sounds produced, but birds may not hear harmonics above 20 000 Hz. Birds apparently hear frequency ranges similar to those heard by humans, except that birds are also capable of sensing very low frequency sounds. Additionally, birds apparently distinguish between separate successive sounds (time discrimination) much better than humans. Because of the sensory differences between humans and birds, caution should be used in interpreting bird sounds and their significance.

Dispersal Sounds

Evolutionary Concepts

It is unlikely that bird sounds exist to satisfy an aesthetic sense in the birds themselves [*59*]. Instead, through an evolutionary process over time, certain vocalizations have elicited specific responses which have increased or decreased the genetic fitness of the caller. Those vocalizations that increase fitness would be selected over ones that did not, assuming everything else is equal. In other words, bird vocalizations generally have a function that relates to the propagation of genetic material and thus to survival [*60*].

Theoretical Aspects

Of all the avian vocalizations that have evolved through time, alarm and distress sounds have shown the most promise for dispersing birds from areas where they cause problems. Feeding calls have been suggested as a method of attracting birds to lure crops or locations where birds can be controlled or tolerated, but this has received little attention [*5,51,61*].

Warning calls are generally of two rather distinct types: alarm and distress calls. Distress calls are given when a bird is restrained or captured [*62,63*] or when a bird is subjected to unfavorable situations such as cold, isolation, or hunger [*51*]. On the other hand, alarm calls generally are given in response to the presence of a predator or an unknown and sudden stimulus [*21,26,37*] or when a bird is excited or angry [*64*]. Starkey and Starkey [*65*] described the characteristics of the alarm and distress vocalizations of mallard (*Anas platyrhynchos*) ducklings and showed that they differed in frequency, duration, and repetition rate. These results demonstrate that distress and alarm sounds can be fundamentally different. The distinction is not as obvious in all birds. Boudreau [*37*] concluded that the starling distress call was actually a modified alarm sound, given both when the bird was in danger and when it was suffering physical distress. Other researchers have reported separate alarm and distress sounds for starlings [*66,67*]. Theoretically, however, distress and alarm calls are distinct sounds initiated by different sets of stimuli.

There is a continuum of sounds that can be considered dispersal calls. Armstrong [*68*] stated that birds may possess a gradation of calls of varying intensity, depending upon the circumstances surrounding the stimulus. He asserted that the nature of the call was influenced by both the internal and external environments and that other factors, such as suddenness of disturbance and stage of the breeding cycle, also contributed. Marler [*69*] summarized this point, noting that "a bird that is alert or nervous is more likely to give alarm when danger threatens than one that is sleepy or relaxed. Thus, the physiological state at the time of the confrontation with a predator cannot be

ignored if we are to understand the process of alarm call production" (p. 53). He mentioned also that the response of another bird to an alarm call would vary according to its physiological state and its previous learning experiences. Fretwell [*70*] and Spanier [*39*] state that residents are more likely to give distress calls than migrants. The logical conclusion from this, then, is that the reaction of a bird to an external stimulus cannot be fully understood without taking its current physiological state and past experiences into account.

Applied Aspects

Some controversy exists over which type of call, alarm or distress, is best suited for bird dispersal work. There are three main areas, however, which affect the utility of these calls as a bird dispersal tool. These are (1) the repellency of the call, (2) the habituation rate, and (3) the effect of regional dialects. Additionally, because the information contained in these sounds generally has biological significance to the receiver, the quality of the broadcast is important and will be addressed later in this paper.

Repellency—Distress sounds have been used as attractants [*71*] and as repellents [*32*]. Thorpe [*51*] noted that "it has been part of the lore of bird snarers from time immemorial that distress squeals of birds held in the hand (particularly young birds) will have a very strong attractive effect on members of the same and sometimes of other species" (p. 21). However, the repellency of a distress call generally is the quality most useful with bird damage problems.

Perrone [*63*] reviewed some of the current hypotheses for a bird vocalizing distress calls when captured. The distress call may be a call for help, a warning to other birds, or a mechanism that could either startle the predator into loosening its grip or bring a second predator to the site, thus increasing the possibility of escape. If the call has evolved as a mechanism to bring help, it may function as an attractant. If the call has evolved as a warning, it may function as a repellent. Similarly, if the call has evolved as a startle mechanism, it may function as neither a repellent nor an attractant. Thorpe [*51*] noted that the distress call of herring gull (*Larus argentatus*) chicks seemed to have no effect on other chicks of the same age, although it may have an effect on the hen. Last, a single distress call may serve more than one function; for example, it may startle a predator and at the same time repel or attract other birds.

Just as there is variability among species in their responses to distress calls (positive or negative attraction), there also appears to be individual variability within a species. Morgan and Howse [*72*] reported that three out of nine jackdaws (*Corvus monedula*) did not learn to avoid or terminate distress calls during behavioral tests. They questioned "whether distress calls act as negative reinforcers for all individuals, and whether they are equally effective negative reinforcers for those individuals which did learn to respond" (p. 489).

Thus, reactions to distress calls vary both with the species and with the in-

dividual. Some species or individuals respond well and others do not [*7*]. Such variation may be a function of previous experience, age, sex, and resident or migratory status.

Alarm sounds have not been tested as thoroughly as distress calls, primarily because true alarm sounds are difficult to record [*34*]. Because alarm calls are given in response to sudden danger, they may trigger an innate reaction in the caller or in other birds nearby [*37,68*]. This reaction may be either to flee or to crouch down and hide [*73,74*]. Thorpe [*51*] cautioned that although some bird calls may be based on a strong innate foundation, there can be considerable leeway for learning in determining a reaction. Accordingly, Marler [*46*] reported that hand-reared chaffinches gave a complete escape response to an imitation of their alarm call heard for the first time, and they also learned to respond to the alarm calls of other species.

Alarm sounds, therefore, at times may be superior to distress sounds for dispersing or repelling birds, assuming that valid alarm sounds exist for the species in question. However, alarm sounds may be more complicated than distress sounds and therefore less predictable [*7*]. Alarm sounds may also need the accompaniment of other vocalizations, such as the attention call preceding the alarm call proper with the herring gull [*5*].

Habituation Rate—Habituation refers to the gradual learning of an animal not to respond to an unimportant repeated stimulus [*42*]. Optimally, a dispersal device such as a warning call should have so definite a biological meaning for the species in question that reactions persist without habituation or else the birds abandon the area altogether [*26*]. This biological meaning can be either from innate programming or from learned experience. Although a bird's physiological state will influence the habituation rate [*37*], it appears logical that the more realistic a broadcast warning sound is, the greater the biological significance and time until habituation. Noise without biological significance should more quickly be accepted as part of the environment. Accordingly, Thompson et al [*75*] reported 8.1 repetitions of distress calls to a starling before habituation, compared with 2.5 to 2.9 repetitions before habituation when using a human voice, escape call, or drug-induced call. Shalter [*38*] found that a recorded human whistle would not elicit a fear response in breeding blue tits (*Parus caeruleus*) even when broadcast at similar sound pressure levels and durations as recorded alarm calls, which provoked immediate responses. Spanier [*39*] reported rapid habituation to recordings of propane exploders by night herons (*Nycticorax nycticorax*), but no habituation to the playback of natural distress calls was observed. Frings [*76*] has stated that "it is not enough just to go out and make a racket. The birds have to be able to recognize this sound" (p. 110). Therefore, it could be expected that birds would habituate to their distress and alarm sounds at a slower rate than to sounds that have no biological significance.

The habituation rate of alarm as compared to distress calls is not clear. Keil (in Ref *26*) reported habituation to playbacks of alarm calls but not to

those of distress calls. In contrast, G. W. Boudreau[3] believed that alarm sounds were effective longer than distress calls because repetitions of the sound are not required as often. Whichever type of call is used, however, it appears that technique is very important for delaying habituation as long as possible. This will be discussed in further detail in a later section.

Regional Dialects—A number of authors have commented on the problem associated with regional dialects [*40, 77–79*]. Vaudry [*40*], in describing bird dispersal work in British Columbia, stated "it must be emphasized that birds will usually respond only to calls of their own species and sometimes only to those from the same region" (p. 11). Marler [*46, 73*], on the other hand, showed that bird alarm calls for many species are similar and have probably evolved in response to selection for both a common language and a call which is difficult to locate. So even though variation in most bird vocalizations is the rule rather than the exception [*44*], alarm sounds of different species show some similarity. Frings et al [*5*] found that the alarm calls of herring gulls were also effective with black-backed gulls (*Larus marinus*) and vice versa. Boudreau [*37*] suggested that regional dialects in alarm sounds were not likely, based on his analysis of resident and migrant birds in the southwest United States. It may be that few regional differences exist between alarm calls, whereas distress calls have observable response differences between populations.

It appears that alarm calls may be more likely than distress calls to elicit responses in a variety of species and in different regions. Birds that encounter other races or species may learn to react to their distress calls [*51*]. However, evolutionary convergence of alarm calls may make them more useful over a larger area.

In general, it appears that both alarm and distress sounds have promise as bird dispersal tools. Alarm sounds may be less likely to result in rapid habituation and may be more likely to elicit a response across species and regions. However, alarm sounds are less tested and are generally more difficult to record. Additional research is needed to find under what circumstances either alarm or distress sounds may be most effective in dispersing birds.

Equipment

The equipment used in a bird dispersal program is important to the ultimate success of the operation [*76, 80–84*]. Brough [*10*] stated that bird recordings and broadcasts should be made with attention to accuracy, signal strength, and clarity. He continued that "any reduction in the standards of any component in the playback equipment is likely to degrade the calls from the level of a recognizable signal to that of a meaningless noise, thus lessen-

[3]Director, Wildlife Technology, Clements, Calif., personal communication, 1981.

ing the response obtained from the birds" (Ref *10*, p. 408). Since the importance of biologically meaningful signals has been emphasized by several authors [*5,10,14,34*], the acquisition of proper equipment should be a first consideration in a bird dispersal program.

It is beyond the scope of this paper to evaluate effectively all of the equipment that has been recommended for use with wildlife recordings. Such an evaluation would require rigorous testing under various environmental conditions, using many different types of equipment. Therefore, only a short review of bird dispersal recording equipment will follow.

Sound energy is transformed by the microphone into electrical energy and then by a tape recorder into a permanent medium on magnetic tape. When broadcasting, the recording is transferred from the tape to an amplifier and then to a speaker, where the electrical energy is transformed back into sound energy. This entire process should be accomplished without any reduction in the integrity or accuracy of the original song or call.

All the components in both the recording and broadcasting systems should have a frequency response that overlaps the frequency range of the song or call of the species in question. In addition, all the components should be matched in terms of their respective specifications.

Microphones

Andrieu [*85*] listed some of the factors that should be considered when choosing a microphone for recording animal sounds. These include the qualities of the sonic field itself or the characteristics of the sound to be recorded, the qualities of the microphone such as voltage output and frequency response curve, and the physical characteristics of the environment such as terrain, surrounding noise, and weather. Fisher [*86*] considers the transformation of sound energy into electrical energy one of the weaker links in an audio system and, therefore, considers selection of a good microphone important. Two types of microphones are often suggested for outdoor wildlife sound recording. These are the moving coil (dynamic) microphone and the condenser (capacitor or electrostatic) microphone [*1,85–89*]. For further information about various kinds of microphones and their relative advantages and disadvantages, refer to Andrieu [*85*], Fisher [*86*], and Simms [*1*].

Reflectors

Parabolic reflectors are commonly used for recording wildlife sounds, but their use should be approached with caution. Many authors have noted problems associated with using parabolic reflectors [*1,48,85,86,90*]. Depending upon the diameter and focal point of the reflector, both high and (especially) low frequencies can be underrepresented in a recording. Parabolic reflectors are useful tools for recording bird sounds. However, an understanding of

their limitations is needed for proper interpretation and application of the sounds recorded.

Recorders

Both reel-to-reel and cassette tape recorders have been used to record bird sounds. Boudreau [*34*] hypothesized that cassette recorders were adequate for birds with lower-pitched sounds, but for birds with higher-pitched sounds, the higher tape speeds associated with reel-to-reel machines were required. Both Fisher [*86*] and Simms [*1*] recognize the ease of using a cassette recorder, but both consider the reel-to-reel recorder more versatile as well as cost- and time-effective. Cassette recordings must be copied onto standard tape before editing and are similar in price to comparable reel-to-reel machines.

For broadcasting bird dispersal recordings, the cassette recorder is a very convenient device to use in the field. Cassettes are easily stored and loaded and do not require detailed instruction for proper use. Kolz and Johnson [*91*] compared the frequency response curve of ten portable cassette recorders in relation to their usefulness with bird dispersal recordings. They rated the recorders primarily according to their ability to reproduce a flat frequency response. Some of the recorders showed good reproductive fidelity between 100 and 12 000 Hz. This frequency range would include the vocal range of most birds in the United States.

Tape

Bird dispersal sounds may be recorded and broadcast using magnetic tapes in reel-to-reel, cassette, and endless-loop cassette formats. The tape selected should be of sufficient quality to produce a good frequency response and low noise [*86*]. It is easy to overlook the importance of tape selection, but Kolz and Johnson [*91*] have commented that "the frequency response obtained from any biosonics system is necessarily limited by its poorest quality component, and it is a common error to overemphasize the frequency response of the amplification system and neglect the quality of the input magnetic tape" (p. 8). In addition, since the thickness of a cassette tape is related to strength, the long, thin C-120 sizes are best avoided [*86*].[4]

Recording and Playback Considerations

Currently, there are no complete or universal guidelines to use when recording or broadcasting dispersal sounds. However, several individuals have reported observations and experiences that should be considered.

Perrone and Paulson [*92*] reported that the percentages of mist-netted

[4]A. L. Kolz, electronic engineer, U.S. Fish and Wildlife Service, Denver, Colo., personal communication, 1981.

birds giving distress calls varied significantly with different handlers. They speculated that the differences in handling may have included "length of time in the net, duration of handling, position of birds during handling or gentleness of handling" (p. 424). Since handlers affect the incidence of calling, they may also affect the intensity or other qualities of the calls. Kuhring [*93*] reported success in eliciting distress sounds from birds by using electric shocks. Further observation and research could help determine whether particular handling procedures, circumstances, or stimuli would elicit a more effective dispersal call.

The elimination of unnecessary background noise from the recording is important for clarity. Background noises can include man-made noises, wind, and electrical noise [*87*]. A windbreak can help eliminate wind noise [*87*], and a microphone held high will help avoid reflection, absorption, or echo problems from the ground [*85*]. A filter that eliminates the lower frequencies may reduce some of the electrical noise [*48*] without adversely affecting the dispersal qualities of the call [*72*]. However, birds sense low frequencies [*53*], so these filters should be avoided unless electrical noise is a problem.

One point that cannot be overemphasized is the importance of good field notes, both when recording a call and when conducting a dispersal operation. Sellar [*94*] gives an example of a standard field note format for use when recording sounds. Playback trials require field notes also. Stockdale [*95*] reported that starlings seemed to respond more to the splice in an endless-loop cassette than to the distress call itself, and G. R. Dudderar[5] believes that a 20 to 30% reduction in resolution may have a greater frightening effect than a more perfectly recorded distress call. R. L. Thompson[6] suggests that hunting calls of avian predators, integrated with alarm calls, may increase their effectiveness, and F. L. Boyd[7] reports that bird dispersal recordings are more effective if they have spaces with no sound instead of continuous noise. Observations such as these should be accurately detailed in field notes to help understand the processes and variables involved.

Guidelines to Field Application

Pretreatment Study

A program for effective bird damage control requires a thorough understanding of the problem and situation. A pretreatment study helps provide the information needed. It should include identification of the problem

[5]Project leader and extension wildlife specialist, Michigan State University, East Lansing, Mich., personal communication, 1981.

[6]Wildlife biologist, U.S. Fish and Wildlife Service, Tallahassee, Fla., personal communication, 1981.

[7]Wildlife biologist, U.S. Fish and Wildlife Service, Nashville, Tenn., personal communication, 1981.

species, recognition of movement patterns, and determination of other parameters such as types of food eaten and sex and age composition. Information from this study would help show why the birds are attracted to the problem area and would facilitate evaluation of various control measures. Evaluation should include a determination of where the problem birds may go if they are dispersed. The direction in which they disperse and their new location are important considerations, particularly at airfields. For example, dispersing birds might fly across active runways, and they might land in another objectionable location within the airfield environment.

In conjunction with a pretreatment study, liability aspects should be established and understood by all parties involved. Additionally, local law enforcement and government agencies should be notified of planned control activities; this will help avoid potential problems such as complaints about noise. Finally, the legal status of the bird species involved should be determined and the appropriate regulatory agencies contacted [*24*].

An Integrated Approach

No single technique for bird damage control is effective at all times in all situations. An integrated approach employs a variety of complementary techniques; this may increase the number of senses stimulated and thereby enhance dispersal efforts as well as decrease the habituation rate. Thus, bird dispersal recordings used in conjunction with other techniques are likely to be more effective than recordings used alone. Murton [*21*] predicted that future developments would center on finding a combination of sound and other stimuli which would lead to the slowest practical rate of habituation while being effective and economically realistic.

Habitat Manipulation—An important first step in many bird dispersal efforts is to remove all sources of food, water, and shelter used by the target species wherever possible [*31*]. This may cause nutritional or other stress for the birds and make dispersal easier. Boudreau [*34*] states that a rule in bird damage control is to look for the bird attractants, then eliminate them if possible. Once the attractant is eliminated, as long as the bird's site tenacity is not too strong, dispersal should be much easier. Therefore, manipulation of the habitat can be an effective management tool, especially when used in conjunction with other dispersal techniques. However, it must be pointed out that habitat modification usually affects other species of vertebrates [*96*]; this should be taken into consideration.

Habitat manipulation can be one or a combination of three types. The first is direct and permanent elimination of certain features that are attractive to birds, such as the elimination of a garbage disposal site [*97*] or watering area [*34*]. The second is the continual management of an area to make it unattractive to the problem species. This may include thinning a roost site [*27, 98*] or managing the height of grass on airfields [*99, 100*]. The third is manage-

ment of an alternate area to make it attractive to birds; using a lure crop [*101*] is an example.

Visual Warning Signals—In his classic monograph on vocal communication in birds, Thorpe [*51*] commented that, although alarm sounds were common, alarm warnings could be communicated by visual cues alone in some instances. This suggests that visual alarm cues may be effective stimuli to use in conjunction with bird dispersal recordings. The combination may decrease the habituation rate and allow enough time to ensure a successful dispersal program [*21,102*]. Busnel and Giban [*9*] speculated that distress calls would be effective at lower volumes if an optical stimulus was added; this would be helpful in urban situations. The optical stimulus can be a model of a bird in a dead or dying position [*103*], a model of a predator [*9*], or other visual frightening device. Pomeroy and Heppner [*104*] found that the mean reaction times of starlings to both light and sound stimuli were very similar (76.38 ms versus 80.64 ms, respectively). Thus, birds may react to all of the alarm stimuli simultaneously, increasing the chance of sufficient fright thresholds for dispersal.

Just as distress or alarm sounds may be more effective the more accurately they are recorded and broadcast, visual cues may also require the same realism. Stout and Schwab [*103*] summarized some earlier work on the dispersal qualities of models of dead gulls, stating that "the visual features had to be almost perfect replicas of the three dimensional appearance of a gull" (p. 4). Models that lacked some visual details were less effective. Similarly, Hardenberg [*105*] reported that stuffed dead gulls displayed a wetted and disordered plumage when exposed to several days of rain, and these birds had a reduced frightening effect on live gulls.

Other Frightening Techniques—Along with audio and visual warning stimuli, other techniques have proven effective in dispersing problem birds [*93,106,107*]. Many of these were listed earlier in this report. One technique often used successfully is pyrotechnics such as shell crackers or fireworks [*40*]. Shooting several birds, where permitted, may be effective when used in conjunction with nonlethal techniques [*102*].

Using Dispersal Recordings

Speaker Location—Vaudry [*40,108*] suggested that speakers be positioned to provide sound over the entire area being protected. The direction and speed of the wind, and the locations of obstacles such as trees that can muffle or block sound, should be considered while placing the speakers [*24,40,109*]. Speakers have been mounted in vehicles, on towers, and on the ground and operated manually, by remote control, and automatically [*31*].

Playback Duration and Interval—Kozicky and McCabe [*14*] noted that the spacing of calls was important to efficacy. Thompson et al [*110*] measured the responses of starlings to broadcast distress calls. They found that

the heart rate peaked within 2 or 3 s and then decelerated. It was also found that a stimulus period shorter than 10 s was effective and suggested that, where habituation is a problem, shorter broadcast periods may extend the effective period of the recording. Langowski et al [*111*] studied the behavioral responses of captive starlings to recorded distress calls. They concluded that an interrupted distress call should be more effective than one played continuously, because starlings habituated rapidly to the continuous playback.

These experiments complement other reports on the proper use of bird dispersal recordings. Vaudry [*108*] suggested that calls be played for 10 s every 8 to 10 min, with the time increasing up to 45 s every 3 min if the bird pressure is great. Boudreau [*34*] reported that bird dispersal recordings should be played intermittently, varying from 5 to 30 s at 3- to 4-min intervals. He mentioned that "a sequence with 10 seconds of sound broadcast every 8 to 10 minutes is adequate for most American pest-bird species" (p. 120). Another report [*31*] advised that the time the tape is playing should be varied from 5 to 60 s, with spaces of 1 to 10 min between playing times. Boudreau [*112*] reported that silent intervals of 3 min or more elicited good responses from horned larks (*Eremophila alpestris*) and house finches (*Carpodacus mexicanus*), whereas the birds often ignored broadcasts of alarm calls played at intervals of 2 min or less. Lucid and Slack [*24*] advised never to run the distress recording continuously. However, Erdman [*113*] advised continuous broadcasting from the time birds were in sight until no birds were approaching. This technique was apparently successful in dispersing mixed flocks of blackbirds from urban roost sites. In general, it appears that the usual technique has been to play the bird dispersal recording for 5 to 60 s at intervals of 3 to 10 min, depending upon how the birds respond.

For dispersal of roosts, the calls should be broadcast as the birds arrive [*24,34,40,109,113*]. The operation should cease when no birds are approaching [*113*] or at dark [*34*]. Roost clearance generally takes more than one evening [*32,34,113*]. For birds such as gulls loafing on a runway or starlings around buildings, the dispersal operation should be continued until the birds do not return to the area [*24*].

Potential Hazards

The loudness of bird dispersal recordings (usually greater than 100 dB) may be disturbing to nearby residents. Both avian and mammalian predators may be attracted by distress or alarm recordings and, in the case of airfields, may increase the problem. Some birds, notably gulls, circle for a while before dispersing [*5,114*] and may cause additional problems to aircraft. Birds moved from one location may cause problems at another. The possibility and consequences of these potential hazards should be evaluated during the pretreatment study and monitored throughout a dispersal program.

Airport Management in Relation to Bird Dispersal Recordings

Several reports have been published concerning bird management on airfields [*8, 17, 21–24, 26, 99, 100, 114–119*]. In general, the normal guidelines for using bird dispersal recordings are also applicable to the airfield environment. A pretreatment evaluation is recommended, and a forecast of what the situation will be five or ten years in the future can be helpful. All bird control work should be coordinated with local law enforcement, government, and other relevant public agencies. Individuals dispersing birds should have direct communication with the flight tower, because dispersing birds may be an immediate hazard to aircraft landing or taking off.

Bird dispersal recordings can be operated by a mobile team that continuously harasses birds near the airfield or by flight control tower personnel who can operate speakers on the airfield by remote control. Records of all bird sightings and attempted dispersals should be coordinated so that daily, seasonal, and yearly activity patterns of birds can be assessed and dispersal attempts evaluated.

Bird dispersal recordings can and have been an effective tool in dispersing birds from the airfield environment. Integrated with other techniques, they should remain a valuable part of bird management programs at airfields.

Concluding Comments

Bird dispersal recordings are not a panacea for all bird problems, but they have proved a valuable tool in integrated bird damage control programs. Kozicky and McCabe [*14*] stated that "the general effectiveness of scare devices is directly proportional to the availability of alternative sources of food and to the proper application of the method . . ." (p. 74). Boudreau [*34*] believed that the failure of bird dispersal recordings to control bird damage is usually traceable to their improper use. Equipment, technique, the quality of the recordings, and the willingness to adapt to a particular situation are all important in determining the success or failure of a bird dispersal operation. Much remains to be understood about bird behavior and dispersal. However, we believe the continuing refinement of bird dispersal recordings and associated techniques can increase considerably our effectiveness in solving bird damage problems.

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Roger W. Bullard,[1] *Edward W. Schafer, Jr.,*[1] *and Richard L. Bruggers*[1]

Tests of the Enhancement of Avian Repellent Chemicals with Sensory Cues

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ABSTRACT: Spray applications of nonlethal repellents are often effective means of protecting maturing agricultural crops from bird damage. Repellents are generally classified as either primary, in which case the animal reacts to the noxious effects of the chemical alone, or secondary, in which case conditioned aversion responses are induced. Secondary repellents more reliably resist high bird depredation of these crops over time. Unfortunately, most pest bird species are not able to detect some secondary repellents at levels which induce conditioned aversion. Thus, higher levels must be used than are actually needed for crop protection. One alternative to this dilemma has been the incorporation of an inexpensive sensory cue (for example, calcium carbonate) into the formulation to replace some of the repellent and reduce crop protection costs. Methiocarb [3,5-dimethyl-4-(methylthio)-phenol methylcarbamate (Mesurol)], currently the most effective avian repellent for field crops, has been the model chemical for testing the action of secondary repellents at the Denver Wildlife Research Center. This paper presents a general review of laboratory and semifield tests used to evaluate various methiocarb-cue (olfactory, tactile, and visual) formulations on both quelea (*Quelea quelea*), a destructive African pest, and red-winged blackbirds (*Agelaius phoeniceus*), a problem species in the United States. Single-choice and paired-preference test methods used on wheat, rice, millet, sorghum, and sunflower in individual cages and group enclosures and conducted under conditions of varying palatability in the alternate food are described. Appropriately designed tests to answer specific questions will lead to further advancements in effectively using chemical repellent-cue combinations for protecting crops from bird pests.

KEY WORDS: vertebrate pest control, birds, repellents, repellent enhancement, sensory cues, preference test methods, alternate foods

Developing chemical repellents to prevent vertebrate pest damage to agriculture and silviculture crops has been a research priority of many laborato-

[1]Research chemists, and research biologist, respectively, U.S. Fish and Wildlife Service, Denver, Colo. 80225.

ries for years. A repellent has been defined by Rogers [*1*] as a "compound or combination of compounds that, when added to a food source, acts through the taste system to produce a marked decrease in the utilization of that food by the target species." He separated repellents into primary repellents, with which the animal reacts to the taste of the repellent alone, and secondary repellents, with which the taste of the repellent becomes a cue to later adverse effects.

Many primary repellents have been tested for protecting agricultural crops from bird damage, but none has produced reliable field results. Naturally occurring tannins in bird-resistant sorghum seeds have probably been the most effective. However, even the most effective varieties may be consumed if bird pressure (high populations or scarce alternative food, or both) is high [*2*]. Topically applied primary repellents have been even less successful. Although Curb[2] has provided some protection under field conditions [*3*], wattle tannin was not effective against quelea (*Quelea quelea*) when applied topically to cereal grain crops [*4*]. In studies with red-winged blackbirds (*Agelaius phoeniceus*) by Rogers [*5*], and black-capped chickadees (*Parus atricopellus*) by Alcock [*6*], only secondary repellents showed promise in high bird pressure situations. Apparently, only toxic, emetic, or nauseating effects of a chemical are sufficient stimuli to control crop damage when bird pressure is high.

Because of the limitations with primary repellents, the failure to exploit the secondary repellent concept, and the time and cost involved in registering new products, only one avian repellent, methiocarb, is widely used. Unfortunately, the chemical is too expensive for foliar use on many low value crops because of the large quantities required for protection. Several studies at the Denver Wildlife Research Center (DWRC) on quelea have indicated that methiocarb caused aversion in birds at levels below which the birds could make taste discriminations [*7*]. Inexpensive chemicals that could be recognized by the tactile, visual, or olfactory senses (wattle tannin, blue dye, and peppermint oil, respectively) were added to formulations, making them effective at lower methiocarb concentrations and thus lower application costs. This paper describes a wide variety of these tests that we used to study cue enhancement of the repellent activity of methiocarb formulations; these tests may be equally applicable to other secondary repellents.

General Procedures

Several basic considerations apply regardless of the test methodology used. Wild captured birds should be acclimatized for at least four weeks in large aviary cages (>20 m^3) before being tested, the photoperiod should be maintained at a constant light-to-dark schedule, and the test birds should be allowed to adapt to any change in the test cages for at least one to two days on their

[2]Synergized aluminum ammonium sulfate manufactured by Sphere Laboratories Ltd., London, Great Britain.

normal ration before the food is changed. Also, the birds should be preadapted to any new food base [*8*], which is accomplished by mixing the new food item into the normal ration in increasing amounts over three or four days.

For individual cage tests on quelea at DWRC, the birds are transferred stepwise from the aviary into 53- by 25- by 31-cm communal cages for at least two weeks and then placed in individual 15- by 23- by 15-cm cages for testing [*8*]. Some gregarious birds like quelea, when tested individually, appear to exhibit less stress when caged next to a bird that they can see [*8*]. With other species (for example, redwings), confinement in small individual cages induces considerable stress and may influence the results of preference tests [*9*]. Enclosure studies with groups of birds thus may help alleviate stress and better approximate normal flock behavior.

Single-Choice Tests

Single-choice tests provide the most rigorous assessment of repellency. The treated food is the only source of nourishment for the test period (usually 18 to 24 h). Because of these rigorous requirements, the Denver Wildlife Research Center and others have used the single-choice R_{50} measurement (the concentration that will repel 50% of the test birds from a food source) as the basic index of avian repellent activity of candidate chemicals [*10,11*]. The repellent qualities of methiocarb were initially recognized through this test. However, this paradigm apparently is not satisfactory for measuring cue enhancement of repellent activity. The food consumption in tests of methiocarb-cue (oleoresins and food colors) combinations was equivalent to that observed from earlier tests with methiocarb alone. The birds simply reacted to the total formulation; the food source and additives were of little influence.

Two-Choice Preference Tests—General

Two-choice preference tests involve the presentation of two food items, under identical conditions, to caged (individual or group) birds. Experience has taught us that the food items should not be placed more than one fourth the width of the cage apart or in a manner that other factors (such as location of perches, water, or doors) could influence the birds' choice. The two food items should be rotated at least daily, and an even number of trials should be conducted (that is, 2, 4, 6, and so on). Shumake et al [*12*] have developed a device that mechanically rotates foods at 1-h, 15-min, or 5-min intervals.

Two-choice preference results can be expressed in a number of different ways, but we usually expressed it as

$$\text{percent preference} = \frac{\text{treated consumption (in grams)}}{\text{treated} + \text{control consumption (in grams)}} \times 100$$

We normally analyze our data with a paired *t*-test to determine the difference between the mean daily consumption of the two foods and with a two-factor analysis of variance (AOV) when several treatments are each tested against a common control. If differences are significant, Duncan's multiple range test [*13*] is used to separate treatment means.

Two-Choice Preference Tests—Equally Preferred Alternate Food

Individual Cage

This paradigm is often used after the single-choice R_{50} method to obtain increased definition of the repellent properties of a chemical [*8*]. Chemicals are initially surface-coated at 0.01 to 0.1% on hulled proso millet seed and tested against the untreated seed on quelea. Only the most active primary repellents will show significant differences between treatment and control ($P \leq 0.05$). Usually at least three of the six test birds have a stereotyped left- or right-handed position preference that a primary repellent at 0.01% will not change. Secondary repellents can be recognized through observing the consumption of treated and untreated food during a six-day test period (Table 1).

It has been our experience with quelea that if a candidate repellent tested at 0.01% elicits nearly complete avoidance for the entire test period, it possibly has secondary repellent properties and produces an adequate sensory stimulus to be detected at low levels. Some plant extracts fall into this category (such as *Agarum caudatum* and *Veronia gigantea* tested at our laboratory). Birds display highly erratic responses after the first day to secondary repellents which are not detectable at low levels because aversion has been associated with the position of the food rather than its taste. There will generally be an overall decrease in total food consumption for at least the second and third test days. For example, in one study the secondary repellent methiocarb without a cue exhibited these characteristics at 0.008% (Table 1). However, when calcium carbonate cue was added, the birds readily avoided the treated food. Thus, by conducting tests using a series of concentrations (Table 1), one can determine the level (0.008% in this example) at which a secondary repellent induces aversion.

Laboratory Enclosure

Quelea—The laboratory enclosure is used to determine group responses to repellent treatments on cereal grain heads [*8*]. In one study five quelea were housed in a 2.5- by 2.4- by 2.2-m screened cage. A hexagonally shaped test apparatus (with 63.5-cm sides), positioned in the center of the cage, served as a simulated "field" for the placement of sorghum heads. Three methiocarb-treated sorghum heads were separated from three untreated heads by a 26-cm

TABLE 1—*Mean percent preference*[a] *of* Quelea quelea *for methiocarb-calcium-carbonate*[b]*-coated wheat seeds in a two-choice test against untreated wheat seeds (individual cage tests, six birds per treatment).*

Treatment Formulation[c]									
Methiocarb, %	$CaCO_3$, %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Overall Mean	*P* Value (*t*-Test)
0.0015	2	56.63	47.26	47.32	56.79	55.92	63.74	54.76	0.302
0.008	2	21.28	21.52	21.17	29.48	22.95	31.47	26.37	0.001
0.016	2	22.74	17.22	9.70	7.52	0.00	7.00	11.09	0.0002
0.024	2	19.94	15.05	0.32	0.46	0.00	1.06	5.06	0.00002
0.008	0	53.76	20.08	56.24	48.61	69.17	36.36	48.94	0.126

[a]The percent preference is the percentage by weight of the treated wheat seeds in relation to the total wheat seeds consumed (treated food consumed + control food consumed = 100 %).

[b]Calcium carbonate used as a cue to enhance the repellent properties of methiocarb formulations.

[c]Respective component or components surface coated on soft winter wheat seeds and tested in paired preference against untreated control wheat seeds.

high by 120-cm wide board, and the positions were alternated daily for six days. The results of a series of methiocarb, cue, and methiocarb-cue studies [*7*] closely resemble the results from the individual cage tests, in that the heads treated with methiocarb and a cue were consistently avoided. The cue-only treatments produced no significant avoidance, and the methiocarb-only-treatment was intermediate, with preference vacillating from day to day.

Red-winged Blackbirds—We conducted another study to determine the level of methiocarb that would reliably repel redwings from ripening sunflower seeds. (It is difficult to apply enough chemical to sunflower seeds in the immature head to induce the conditioned-avoidance response in birds [*14*].) Immediately before the test, we applied each treatment using a calibrated spray table to fresh sunflower heads, and the excess moisture was removed with fans. Then two treated heads, two untreated heads, and a feeder containing Purina Layena, were placed in 1.2- by 1.2- by 1.8-m enclosures (four replications) containing four birds each. Damage (in square centimetres) was read by template [*15*] at 21 h. One-tailed paired *t*-tests of the data ($P \leq 0.05$) indicated that 8.4 kg/ha was the lowest level that would elicit avoidance (Table 2). It would have been desirable to have used more replications, as indicated by the 2.8-kg/ha results. The enhancement effect of cues is illustrated by the percent preference for the 5.6-kg/ha and 16.8-kg/ha treatments compared with and without cue. Damage to the methiocarb-cue-treated heads was lower in both tests. An equation regressing preference on kilograms per hectare of methiocarb was significant ($P = 0.00002$); however, there was no significant correlation between the total damage and the alternate food consumption. This method appeared to be more appropriate than the less-preferred alternate-food method discussed in the section on laboratory enclosures using sunflowers.

TABLE 2—*Paired preference (equally preferred alternate food) enclosure test of methiocarb-cue protection from red-winged blackbird damage to sunflower heads.*

Methiocarb, kg/ha	$CaCO_3$,[a] kg/ha	Alternative[b] Food, g	21-h Damage, cm^2		Percent[c] Preference	*P* Value (Paired *t*-Test)
			Treated	Control		
16.8	56.1	19.0	85	722	10.5	0.015
8.4	56.1	79.0	230	650	26.1	0.003
5.6	56.1	23.0	305	745	29.0	0.091
2.8	56.1	69.0	355	560	38.8	0.002
0.0	56.1	38.6	400	410	49.4	0.470
16.8	0	13.0	140	488	22.3	0.012
5.6	0	31.0	410	385	51.6	0.575

[a]Calcium carbonate used as a cue to enhance the repellent properties of methiocarb formulations.

[b]Purina Layena poultry mash provided in the poultry feeder in the center of the cage.

[c]The percent preference is the percentage in square centimetres of damage to the treated sunflower heads compared with the total damage (treated sunflower damage + untreated sunflower damage = 100%).

Two-Choice Preference Tests—Less Preferred Alternate Food

By altering the palatability of the alternate food, one has another means of investigating the repellent properties of a chemical. Rogers [*5*] used this less-preferred alternate food approach in his early investigations of the secondary repellent properties of methiocarb. This method involves treating a preferred food and choosing a less preferred food as the alternate food. Thus, one can "titrate" the activity of a secondary repellent by manipulating the acceptability of the alternate food or by prescreening for birds that have distinct preferences for one food over another.

Individual Cage

Garrison [*16*] preselected quelea that preferred hulled proso millet ($\geq 70\%$) to Martin X sorghum in two-choice preference tests. These birds were then tested on a decreasing series ($3.33\times$) of methiocarb-millet formulations against the sorghum (Table 3). The 0.0013% concentration elicited some change in preference, and the 0.0045% level caused nearly a complete switch to the nonpreferred sorghum. Garrison used this method to obtain an R_{50} index of 0.0015% which is ten times less than had been obtained earlier on quelea given no choice [*10*].

The same procedure was used to test wattle tannin (a primary repellent) on prescreened quelea [*4*]. Untreated millet was significantly preferred ($P \leq 0.05$) over 0.2% coated millet, but, with sorghum as the alternate food, tannin levels as high as 0.68% tannin did not induce repellency.

TABLE 3—*Consumption of methiocarb-treated millet and nontreated sorghum grains by caged quelea (individual cage tests, two choices with less-preferred alternate food).*[a]

Number of Birds	Sex	Treatment Concentration	Total Food Consumption, g	
			Millet	Sorghum
3	♂	0.015	1.0	20.2
3	♀		1.2	24.0
3	♂	0.0045	1.7	29.0
3	♀		3.1	32.4
3	♂	0.0013	23.5[b]	11.9
3	♀		23.9[c]	10.4
3	♂	0.00039	30.0	5.9
3	♀		34.7	3.8

[a]Courtesy of M. V. Garrison of the Denver Wildlife Research Center.
[b]One male consumed more nontreated sorghum than treated millet.
[c]One female consumed more nontreated sorghum than treated millet.

Laboratory Enclosure

This paradigm was tested as an alternative to the equally preferred alternate food method discussed earlier for determining the level of methiocarb that repels redwings from sunflowers. Two treated heads and Purina Layena poultry mash were placed in the enclosures (four replications) containing four birds each. Sunflower head damage and alternate food consumption at 21 h were measured (Table 4). The most obvious effect was methiocarb related. Twice as much damage occurred with untreated heads as with either 5.6- or 11.2-kg/ha treatment and 4.67 times as much damage with the 16.8-kg/ha treatment. Alternate food consumption had little measurable influence except possibly for the untreated head tests where no conditioned aversion occurred. Birds ate much higher quantities of sunflower, in this test, leaving less stomach capacity for the less preferred alternate food. An equation regressing square centimetres damage on kilograms per hectare of methiocarb was significant ($P = 0.01$), but it is obvious that this method is less sensitive than the equally preferred alternate food method for measuring small differences in methiocarb activity and consequently is less useful for the intended objective of detecting the lowest effective methiocarb concentration.

Field Enclosure

Methiocarb-cue studies on quelea have been conducted in enclosure studies of standing crops in Africa [7]. A 1- by 1- by 2-m (2-m^3) screen cage was placed over stands of sorghum or millet. Ten quelea were placed in each cage after the chemical application. Millet and sorghum grain (less palatable than the immature crop seed) were available as alternative food in containers suspended

TABLE 4—*Paired preference (less preferred alternate food) enclosure test of methiocarb-cue protection from red-winged blackbird damage to sunflowers.*

Methiocarb, kg/ha	$CaCO_3$,[a] kg/ha	Sunflower Damage, cm^2	Alternate Food Consumed, g
0	0	1714	58.0
0	56.1	1081	37.5
0	112.2	1388	85.0
5.6	0	470	44.3
5.6	56.1	751	128.0
5.6	112.2	863	109.0
11.2	0	485	58.0
11.2	56.1	602	111.0
11.2	112.2	914	92.0
16.8	0	275	84.0
16.8	56.1	340	81.0
16.8	112.2	280	101.0

[a]Calcium carbonate used as a cue to enhance the repellent properties of the methiocarb formulation.

[b]Purina Layena provided in the poultry feeder in the center of the cage.

from the cage tops at head height. In one study, three replications of 0.5% methiocarb plus 1% tannin were applied to both millet and sorghum plants in the dough stage. An identical set of cages was sprayed with 1.0% methiocarb, and a third set was left untreated. A significant treatment effect was detected for both cereals ($P \leq 0.001$; Kruskal-Wallis test) in that untreated sorghum and millet received significantly greater damage than either of the two treatment groups. Also, for both cereals there were no differences in damage between the two treatments. Similar results were later obtained in identical enclosure studies on wheat [*7*].

Method Combinations

Shumake et al [*17*] combined some of these principles in a more sophisticated study of the relative importance of taste versus visual feeding cues that could become associated with methiocarb exposure. Groups of quelea were preexposed to the specific stimuli in two-choice preference tests (that is, blue versus green; sweet versus sour) during the first week. During Week 2, the birds in each group were exposed every other day to 0.018% seeds treated with the color or taste that had been preferred during Week 1. The groups were then given three 18-h choice periods identical to the preexposure period each week. The changes in paired-preference patterns, influences of differences in food palatability, and salience of conditioned aversion effects were measurable factors by this paradigm.

Discussion

The choice of a test method for assessing specific properties of a repellent is extremely important. If it is a rigorous test of overall food protection potential, then the single-choice R_{50} index method is probably appropriate. If one wishes to define the mode of action of the repellent, then the equally preferred alternate food paradigm is appropriate. However, concentrations must be kept low (ten times lower than the single-choice method) so that relative activity can be compared among chemicals. Subsequent tests with repellent-cue combinations would further clarify these differences.

The choice of conditions for using the less-preferred alternate food paradigm is more difficult to establish but can be extremely important in determining a specific chemical concentration that elicits aversion. One must carefully choose the alternate food to generate the appropriate preference differential for the intended objective. Shumake et al [*17*] only intended to establish a methiocarb level that would readily induce conditioning and therefore used the single-choice R_{50} method. However, Garrison [*16*] wanted to know the lowest level at which methiocarb elicits conditioned aversion, used slightly less preferred sorghum as the alternate food, and obtained a tenfold lower R_{50}. A wider preference differential (sunflower heads versus poultry mash) did not have as much sensitivity for detecting aversion as did the equally preferred alternate food method.

With the tremendous costs involved in registering new crop protection chemicals, it is unlikely that many new repellents will be developed. It is imperative that we find ways to make those currently available more effective. This may be through innovative application methods [*1*] or with additives such as sensory cues. As we learn more about the behavioral, physiological, and ecological aspects of a pest species, and begin to ask the proper questions and design the appropriate tests to answer them, further advancements toward effectively using repellents to protect crops will be made.

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David L. Otis,[1] *Nick R. Holler,*[2] *Paul W. Lefebvre,*[2] *and Donald F. Mott*[3]

Estimating Bird Damage to Sprouting Rice

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ABSTRACT: Sampling procedures are described for assessing bird damage to sprouting rice. Optimum sampling schemes are derived for both large-scale surveys and controlled experimental research from data collected from a 1980 controlled experiment and a 1981 survey. A basic recommendation is that each field be sampled by using a stratified or systematic design, depending on the objective of the specific study. Sampling points consist of paired plots, one of which is protected from bird damage by a wire exclosure, that are located on transects placed randomly across the field. Methods are derived for calculating the number of plots per transect and the number of transects necessary to achieve a given level of precision or to maximize precision for a given cost. A stratified design is also proposed for conducting large-scale surveys, and recommendations are given concerning the required number of fields for a desired precision.

KEY WORDS: sprouting rice, survey sampling, damage assessment, vertebrate pest control, Louisiana, blackbirds

Blackbirds have been damaging sprouting rice since the time rice culture was initiated in the United States [*1*], and research on this problem has been conducted periodically for nearly 50 years [*2–5*]. Problem-defining large-scale damage surveys, as well as experiments that test treatment forms for efficacy, require valid and efficient sampling designs for producing estimates of loss, which can be expressed either as the percentage of the sprouts re-

[1]Research statistician, Denver Wildlife Research Center, U.S. Fish and Wildlife Service, Denver, Colo. 80225.

[2]Wildlife biologists, Denver Wildlife Research Center, U.S. Fish and Wildlife Service, Gainesville, Fla. 32601.

[3]Wildlife biologist, Denver Wildlife Research Center, U.S. Fish and Wildlife Service, Bowling Green, Ky. 42101.

moved or as the total sprouts removed, the basic difference between the parameters being that the percentage loss does not account for the field size.

Most methods for estimating sprout loss to birds have been dependent on counts of sprouts in small plots or quadrats. These counts are conducted two or three weeks after planting, when the sprouts are about 8 cm high and no longer susceptible to bird damage. Besser [*4*] and Mott et al [*5*] used counts of surviving seedlings in 0.093-m^2 (1-ft^2) circular plots to evaluate control techniques. This approach has two potential drawbacks: (1) an estimate of percentage loss in the experimental unit is not directly available, and (2) differences in potential yield among experimental units are not accounted for. Later, Mott et al (unpublished data), in addition to establishing 50 randomly located 0.093-m^2 plots in each sampled field, also used 25 independently located 0.093 m^2 exclosed plots in each field to avoid the aforementioned difficulties. Although this sampling design produced improved, essentially unbiased estimates of both total and percentage sprout loss, sampling variances of the estimates were relatively large.

Holler et al [*6*] improved on Mott's sampling design as follows. Each rice field (experimental unit) was divided into five strata of equal width and, within each stratum, two transects were randomly located perpendicular to the baseline (Fig. 1). A total of 50 paired sampling points were allocated to the strata in proportion to their area. The points for each stratum were then randomly placed along the two transects, the number of points on a transect depending upon the relative lengths of the transects in that stratum. At each

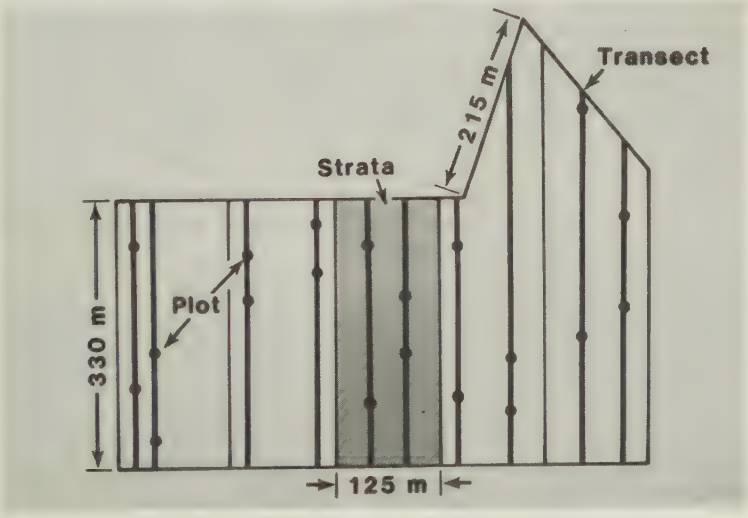

FIG. 1—*Allocation in a stratified sampling design for a fixed cost of 11 man-hours.*

point, two circular 0.186-m^2 (2-ft^2) plots were established a distance of 1.8 m (6 ft) apart, with one of the plots protected by a birdproof wire screen exclosure. Paired counts of sprouts in the protected and unprotected plots were then used to estimate the percentage loss as well as the total number of sprouts removed.

This sampling design was also used in a 1981 survey of bird damage to sprouting rice in Acadia and Evangeline parishes, Louisiana. The objectives of this survey were twofold: (1) to obtain baseline data on variation in damage among fields in two areas of traditionally high bird damage and (2) to obtain data on optimum plot size, spacing, and placement. The objective of this paper is to use the 1981 survey data, supplemented by the data of Holler et al [*6*], to make recommendations for the construction of efficient sampling designs for estimating either total absolute sprout loss or percentage sprout loss, in both experimental and large-scale survey situations.

Procedures

1981 Damage Survey

In both Acadia and Evangeline parishes, Louisiana, 50 farmers in each parish were randomly selected from complete lists of rice growers supplied by the Agricultural Stabilization and Conservation Service and the Cooperative Extension Service. The 50 growers in each parish were contacted by telephone, in order of their selection, until 10 cooperators were found whose first-planted field was to be between 4.047 and 40.47 ha (10 and 100 acres). Fifty sampling points were chosen in each field as earlier described. At each point, two 0.186-m^2 plots, 0.43-m (1.4-ft) square, were established a distance of 1.8 m apart. The selected location on the transect was randomly chosen to be either protected or unprotected, and the other plot was then placed along a randomly selected bearing in relation to the transect. Exclosures were placed in the fields immediately after seeding and, in water plantings, before draining of the field had begun. Also, in one field in each parish, an additional unprotected plot was established at a distance of 0.9 m (3 ft) from the protected plot, along the same relative bearing.

Intuitively, we know that the more highly correlated the natural sprout density between the protected and unprotected plot is, the better the damage estimate will be, and thus we wanted to collect data that would allow us to determine if estimates from plots 0.9 m apart were more precise than those from plots 1.8 m apart. If no real difference exists, we prefer the greater distance, because it decreases the chance that the exclosure may have an influence (either frightening or attracting) on bird behavior, which in turn could lead to biased estimates. Finally, for each 0.186-m^2 plot, a separate sprout count was recorded for an included 0.093-m^2 subplot. This information was

intended to allow determination of whether use of the smaller plot, which obviously would require less sampling effort, produced estimates equal in precision to those from the larger 0.186-m^2 plots.

The choice between the two plot sizes and the two intervals between paired plots was determined by comparing the sampling variances of the estimators of total sprout loss. The sampling design used was equivalent to stratified random sampling, with two-stage (transect location, plot location along a transect) sampling within the strata. The appropriate formulas for estimating total loss and percentage loss and the corresponding variances are given by Cochran (Ref *7*, pp. 303–318).

Determination of Within-Field Sampling Design

In developing optimum sampling schemes for estimating field loss, we will be operating under the following assumptions: (1) sampling points consist of paired plots with one of the plots protected from bird damage, and (2) points are randomly located on transects placed at random perpendicular to the baseline of the field. At present, we also assume that the field has been stratified into areas of equal baseline width, as described previously. (Systematic, rather than stratified, designs will be discussed later.) In this context, the creation of optimum designs depends only on specification of the number of transects per stratum and the number of plots per transect. The approach used in choosing such sampling intensities involves minimizing the variance of the estimator for a given cost or, equivalently, minimizing cost for a specified variance. That is, if a given amount of manpower is available for sampling a field, we want to know how best to allocate this manpower; vice versa, if a given level of precision of the estimator is desired, we want to know how the manpower needed to produce this level of precision can be minimized. These questions can be answered by using the appropriate variance formulas, a specified cost function, and the method of Lagrangian multipliers (Ref *7*, pp. 313–316). Refer to the Appendix for details.

Determination of Damage Survey Requirements

In a controlled experiment the number of fields (experimental units) necessary to achieve the researcher's goal usually depends upon the expected magnitude of treatment differences, significance level desired, and so on [*8*]. If, however, the objective is to produce an unbiased, relatively precise estimate of loss over a large geographical area, then one of the essential parameters to be determined is the number of fields that are necessary for a specified level of precision. The necessary number may be in terms of the total for the survey or in terms of fields per stratum, if the area, say a state, has been geographically stratified into counties, for example. In a large-scale survey of sprouting rice damage, percentage loss rather than total sprout loss is usually

the response of interest, and, therefore, the estimators corresponding to this response are used in determining the optimum sampling design. For illustrative purposes, we shall be concerned with constructing a sampling design for a statewide survey of sprouting rice loss, with the counties (parishes) of the state as the strata. Therefore, specification of the necessary number of fields to be surveyed per parish is of primary concern. The basic survey design involves stratified random sampling, treating parishes as strata and fields within strata as the sampling units.

Results

Plot Size and Spacing

The estimates of the average total sprout loss per field in the 20 fields sampled in 1981 were $\hat{T}_2 = 3\,616\,664$ and $\hat{T}_1 = 3\,846\,218$ sprouts for the 0.186-m^2 and 0.093-m^2 plots, respectively. These averages were not significantly different ($t = 0.09$, $P = 0.93$), although the difference between the two estimates in a given field was often very large, reaching as high as 13 million sprouts in one field in Acadia parish. With respect to the precision of the two estimators, $\hat{T}_2$ and $\hat{T}_1$, the average sampling variances of the 20 estimates were 2.24×10^{13} and 4.20×10^{13}, respectively. Thus, on the average, $\hat{T}_2$ was roughly twice as precise as $\hat{T}_1$.

It could be reasoned that it takes twice as much time to count sprouts in a 0.186-m^2 area than in a 0.093-m^2 area, and therefore twice as many of the smaller plots could be sampled, thus achieving roughly equal precision. However, considering that the actual time spent counting sprouts is relatively minor compared with the time and effort required to set up the sampling design, to construct and carry exclosures into the field, to walk the transects, and so on, it seems logical that the larger plots are preferrable. Therefore, in all subsequent analyses, data from 0.186-m^2 plots were used.

Although the data for comparing plots placed 1.8 m versus 0.9 m apart were minimal (recall that such data were collected in only one field in each parish), there was no evidence of any real difference in the precision of the two estimators. In fact, the variance of the estimator based on plots farther apart averaged 40% less than the estimator using plots 0.9 m apart, although we hesitate to place any confidence in the consistency of this relationship because of the small sample size. On the basis of available data, we therefore concluded that use of 0.186-m^2 plots 1.8 m apart produced the most precise estimates of those investigated.

Within-Field Sampling Design

Determining field sampling design depends, in part, on specifying the cost factors c_1 and c_2 or, more specifically, the ratio c_1/c_2, as shown in Eqs 2 and

3 of the Appendix. Here, c_1 represents the total time required, per transect of average length, to initially walk the transect when the plots are established, plus the time required to walk the transect and remove the exclosures at the time of actual sampling. The cost factor, c_2, represents the time required initially to establish the location of the plot, plus the actual time spent counting sprouts at the time of assessment. Based on the damage assessments conducted in 1980 and 1981, the values of 48 and 6 min were chosen for c_1 and c_2, respectively.

Total Sprout Loss—If the objective is to estimate total sprout loss or, equivalently, mean sprout loss per unit of area, the Appendix provides the basic relationship $\bar{r} = 0.0033\bar{R}$, where $\bar{r}$ is the number of sampled plots per transect, and $\bar{R}$ is the total number of potential plots on the transect. For example, the number of plots on a transect of length 122 m (400 ft), 183 m (600 ft), 244 m (800 ft), and 305 m (1000 ft) should be one, two, two, and three, respectively. To illustrate completion of the sample allocation, the field pictured in Fig. 1 will be used. The average number of possible plots per transect in the field is roughly 850 (= 366 m/0.43 m) so that $\bar{r} \approx 3$, and, using Eq 1 in the Appendix, we find that each transect "costs" roughly 1 h (66 min), remembering that this cost function does not include time required to obtain field dimensions, select random numbers, and so on. If five strata were used, then roughly 11 h would be needed to establish two transects in each stratum ($2 \times 5 \times 66 = 660$ min = 11 h) with an average of three plots per randomly chosen transect. Figure 1 depicts this sampling plan with specified randomly chosen transects and plot locations.

If calculation of the necessary cost for a fixed level of precision is desired, estimates of the necessary sampling variances can be substituted into the formula for the variance of the estimator of total loss (or average loss per plot) to derive the appropriate relationship. For illustration, the estimator for average loss per plot shall be used. By using variance estimates calculated from our 1980 and 1981 data, and Eq 4 in the Appendix, Table 1 was created. It presents the necessary number of transects and the resulting cost required for specified levels of precision. To assist in interpretation, we note that the average number of sprouts in a 0.186-m^2 exclosure is roughly 50.

TABLE 1—*Cost, C, in man-hours, and number of transects, n, required to achieve a given standard error for mean sprout loss per plot, for the example field in Fig. 1.*

Standard Error	*C*	*n*
5	139	126
7	70	64
10	35	32
15	15	14
25	6	5

Percentage Sprout Loss—If the parameter of interest is percentage loss, rather than number of sprouts lost, the Appendix shows that the optimum allocation is $\bar{r} = 0.4808\,(\bar{R}/N)$, where $\bar{r}$ and $\bar{R}$ are defined as before and N is the number of potential transects in a stratum. For example, if the field were square and stratified into five equal areas, $\bar{R}/N = 5$, resulting in $\bar{r} = 2.4$ or, rounding up, three plots per transect. For our example field in Fig. 1, we see that $N = 125/0.43 = 290$, and therefore $\bar{r} = 0.0017\bar{R} = 0.0017(850) = 1.4$, implying an average of two plots per transect, if the next higher integer is used. If cost C has been fixed, then the cost function in the Appendix shows that 1 man-hour is required for every transect. The resulting allocation is obviously very similar to that arrived at using the procedure for estimating total sprout loss. This allocation suggests slightly fewer plots per transect on the average, and a corresponding slight increase in the number of transects.

The data indicate that as percentage loss increases, the variance of the estimator decreases. Therefore, calculation of necessary cost and sample allocation for a desired level of precision must be conditioned upon a fixed level of damage. Table 2, created from calculations given in the Appendix (Eq 5), il-

TABLE 2—*Number of transects,* n, *(equaling the number of man-hours) required to achieve a given standard error (SE) for percentage loss, using the field in Fig. 1 and differing values of expected loss,* P.[a]

P, %	S_{bp}^2 (10^{-5})	S_{wp}^2 (10^{-4})	SE	*n*
5	33.0	29.0	1	574
			2	144
			5	23
			10	6
10	31.0	27.5	1	541
			2	135
			5	22
			10	5
25	28.5	24.0	1	492
			2	123
			5	20
			10	5
50	19.0	17.0	1	332
			2	83
			5	13
			10	3
75	10.0	10.0	1	179
			2	45
			5	7
			10	2
90	5.0	7.0	1	98
			2	24
			5	4
			10	1

[a]The average number of plots per transect is two. (The values for S_{bp}^2, the variance in sprout loss among transects within a stratum, and S_{wp}^2, the variance among plots within a transect, were those used in Eq 5 of the Appendix.)

lustrates the principle that the effort required to achieve a given level of precision increases as damage in the field decreases. For losses below 25%, a substantial amount of time is required to produce reasonably precise estimates. However, if sprout loss is on the order of 75%, only 7 manhours are required to produce a very small standard error of 5%.

Large Area Survey Design

To estimate percentage or yield loss due to birds damaging sprouting rice over a large area, a design is required that specifies the number of fields needed and how they are to be selected and sampled.

As an example, consider the problem of estimating the percentage loss in the twelve parishes of Louisiana that contain about 90% of the acreage planted to rice. Rather than select fields completely at random from this area, each of the parishes shall be treated as a stratum so that sampling is ensured in each of the parishes. Within each parish, a specified number of fields will be selected at random, in proportion to the number of acres of rice planted in that parish. Within the framework of such a stratified sampling design with proportional allocation, an appropriate field sampling design as well as the number of fields to be sampled need to be determined.

The contribution of the variance of an estimate of percentage loss for a given field to the overall variance of the percentage loss estimate for the entire area is negligible compared with the contribution due to the variance in loss among fields within a parish. For this reason we choose not to sample individual fields heavily but rather to devote the most effort to increasing the number of fields sampled. Because we feel that an individual field estimate should have a standard error at least as small as 10% in most situations, an arbitrary choice of four transects per field with an average of two plots per transect has been made for purposes of discussion, based on the results given in Table 2. (Note that this choice implies use of no more than four strata in a field. An alternate method for locating plots is proposed later.)

The Appendix (Eq 8) illustrates that calculating the required number of fields for a given precision of the overall survey estimate depends on the variation in damage among fields within a stratum. Using the results from the 1981 survey in which the average loss was low (5%), and from control fields in the 1980 study in which the average loss was high (80%), reasonable values for among field variation were chosen for some different survey situations. Table 3 presents the resulting required number of fields corresponding to specified standard errors and illustrates the fact that required sampling effort depends heavily on the characteristics of the fields being surveyed. Of course, it is impossible to know exactly what to expect, in terms of the magnitude and variation of loss, before the survey is conducted. However, it is important that the researcher use all the biological as well as statistical evidence available to help guide the choice of a survey design.

TABLE 3.—*Required number of fields for a specified standard error (SE) of the percentage loss estimate of sprout loss in the rice-producing parishes of Louisiana.*[a]

SE	Expected Damage Situation			
	I ($S_w^2 = 0.0064$)	II ($S_w^2 = 0.025$)	III ($S_w^2 = 0.0625$)	IV ($S_w^2 = 0.16$)
0.01	64	256	625	1600
0.02	16	64	156	400
0.05	3	10	25	64
0.10	...	3	6	16

[a]The expected survey situations are as follows: I: low (5%) average loss with small variation among fields (0 to 25%); II: low (5%) average loss with medium variation among fields (0 to 60%); III: high (50%) average loss with medium variation among fields (40 to 100%); IV: moderate (25%) average loss with maximum variation among fields (0 to 100%). (The values given for S_w^2, the variance in damage among fields within a parish, were those used in Eq 9 of the Appendix.)

In terms of required manpower costs for the large-scale survey, recall that we have estimated that 4 man-hours per field will be needed (Table 2) for the sampling design previously chosen. However, this cost covers actual sampling time only and does not account for time needed for travel and planning required in conducting any survey. These added costs could easily double the cost per field, so that, for example, a survey of 100 fields might require as much as 800 man-hours.

Systematic Sampling

All of the preceding material has been developed within the framework of a stratified design for sampling damage within selected fields. An alternative to stratification is the use of a systematic sampling design for placing plots within a field. To illustrate this method, consider the example field in Fig. 1, and suppose that it is desired to place 20 plots along four transects in the field. The first step is to divide the length of the baseline, 625 m, by the number of desired transects to arrive at the intertransect spacing, about 156 m. Next, a random number between 1 and 156 is chosen and the first transect is located at this distance from one edge. The remaining transects are located at intervals of 156 m from the first transect. Thus, for example, if the random number chosen is 62, then transects are located at 61, 217, 373, and 529 m. The transect lengths will average about 366 m, and, therefore, the 20 plots need to be located within a total of 1464 m of transect. This total length is divided by the number of plots to arrive at an interplot distance of 73 m. A second random number between 1 and 73 is chosen, and the first plot on the first transect is placed at this distance down the transect. Subsequent plots are located a distance of 73 m apart. When the researcher reaches the end of a transect, the distance count is simply continued on to the

next transect. For example, if the number chosen is 30, plots on the first transect are placed at 30, 103, 176, 249, and 322 m, assuming an average transect length of 366 m. The first plot on the second transect, assuming the researcher turns around and comes back down toward the baseline, would be located at 30 m [73 − (366 − 323)]. Figure 2 illustrates the location of plots using this systematic design.

This method can be very attractive because it is somewhat easier to set up than stratified sampling (only two random numbers are required), while ensuring uniform coverage of the field. However, unbiased variance estimators for the estimators produced are not available, unless the researcher is willing to make some assumptions concerning the distribution of damage in the field [7,9]. Earlier, we alluded to the fact that, in two-stage sampling, the contribution of the variance among second-stage units is negligible compared with the magnitude of the primary unit contribution. Therefore, a slight bias in the estimator of variance among second-stage units can be tolerated, because the bias will contribute little to the overall estimate of the variance of the estimated sprout loss. In place of an unbiased estimator, the usual estimator of variance assuming simple random sampling of second-stage units can thus be used, if it is desirable to sample the second-stage units systematically. The definition of this unit depends on the overall sampling design. In a large-scale survey such as the one just described, the primary unit within a stratum (parish) is a field, and therefore the entire field could be systematically sampled as in the earlier example. If, on the other hand, fields are being us

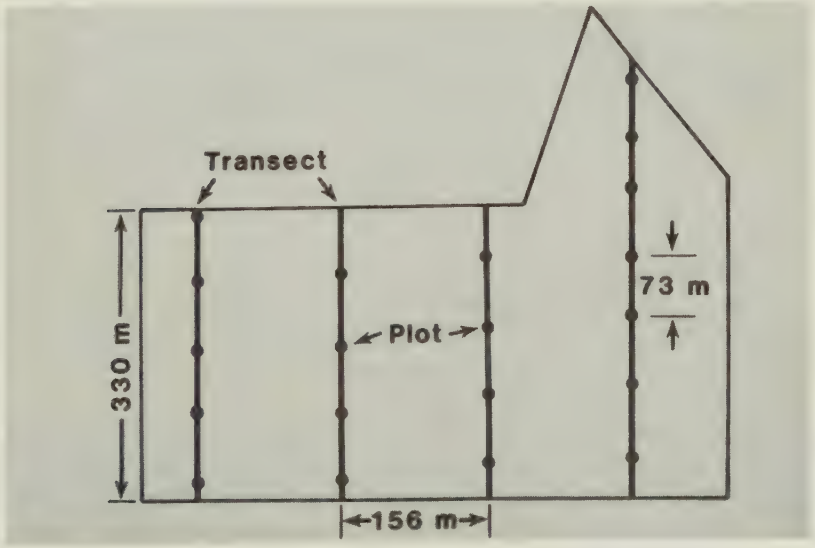

FIG. 2—*Placement of 20 sampling plots in a systematic sampling scheme with four transects.*

as experimental units in a controlled experiment [6] so that the variance of individual field estimates of loss is of more importance, use of systematic sampling for specifying transect locations is not recommended. In an experimental situation, the transects within the field are really the primary units, and the plots within transects represent the second-stage units. Thus, if the systematic methods are to be used at all, it is best to use them only for locating plots along a transect. That is, we recommend that the field be stratified and that, within each stratum, the transects be located completely at random. The researcher may then use either random sampling or systematic sampling to locate plots on a transect, and the variance formula assuming simple random sampling at all stages can be used.

Discussion

General Recommendations

From the preceding results, it is obvious that there is no one best sampling design for either an experimental or large-scale survey situation. Optimum sampling designs have been shown to be a function of such parameters as manpower costs, variation among plots within a transect, variation among transects within a stratum, variation among fields within a county, transect length, and stratum width. However, we can make some very general recommendations on sampling design based on our specific results, but the reader should realize that these generalities must be adapted to the specific survey or research situation.

Large-Scale Survey—If the objective is to estimate average loss over a large area, the area should first be divided into small geographical areas that can be treated as strata. The strata boundaries could be either political (county) or based on physiographic features of the area. If the researcher has no clue as to what kind of damage situation is likely to be encountered, Table 3 suggests that a minimum of 100 fields should be surveyed if reasonably precise estimates are desired. The number of fields per stratum should be allocated in proportion to the acreage of rice in the stratum. For each randomly located field within a stratum, either systematic or stratified sampling can be used to locate the paired sampling points along at least four transects. The number of plots per transect should be proportional to the length of the transect, with an average of two to three per transect. A minimum of ten sample points per field should be used.

Controlled Experimentation—If the objective of the research involves a statistically designed controlled experiment, then much more precision in the individual field loss estimates is required. We recommend stratification of the field into at least four geographic areas, preferably of equal width, as described previously. The number of plots per stratum should be based on stratum size, and the number of plots per transect should be proportional to

transect length. Within each stratum, at least two transects should be randomly located and an average of at least three paired sampling points established per transect. The locations along a transect can be chosen either at random or systematically.

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APPENDIX

Within-Field Sampling Design

The cost function used throughout this paper is of the form

$$C = c_1 n + c_2 n\bar{r} \tag{1}$$

where

C = total time (man-minutes) available,
c_1 = cost per transect, minutes,
c_2 = cost per plot, minutes,
n = number of transects, and
$\bar{r}$ = number of plots sampled on a transect of average length.

Because the variance expressions for the estimators of total loss and percentage loss are different, the formulas for determining optimum allocation will differ. For total loss, the key relationship is

$$\bar{r} = \bar{R}\frac{S_{wt}}{S_{bt}}\sqrt{\frac{c_1}{c_2}} \tag{2}$$

and for percentage loss the expression is

$$\bar{r} = \frac{\bar{R}S_{wp}}{NS_{bp}}\sqrt{\frac{c_1}{c_2}} \tag{3}$$

where

$\bar{R}$ = average number of potential plots (= length of average transect/plot length) per transect,
N = number of potential transects in the stratum (= stratum width/plot width),
S_{bj}^2 = variance in sprout loss ($j = t$) or percentage loss ($j = p$) among transects within a stratum, and
S_{wj}^2 = variance in sprout loss ($j = t$) or percentage loss ($j = p$) among plots within a transect, weighted by transect length.

In the derivation of these formulas, it was assumed that the number of sample plots per sampled transect is proportional to the length of the transect.

By substituting estimates of the aforementioned parameters calculated from the 1980 and 1981 data into Eqs 2 and 3, the average number of plots per transect can be determined for the estimators of total and percentage loss respectively. Given a value of $c_1/c_2 = 8$ (see the main text), the ratios $\gamma_t = S_{wt}/S_{bt}$ and $\gamma_p = S_{wp}/S_{bp}$ are of primary importance. It was possible that the value of either of the ratios could change with the level of damage in the field. Therefore, γ_t was plotted against the average number of sprouts lost per plot for each of the 140 strata sampled in 1980 and 1981, and a plot was also made of γ_p versus the percentage loss. In both instances, no evidence of a relationship was apparent, and nonsignificant ($P > 0.10$) simple linear regression coefficients support this conclusion. As a result, an average value of $\gamma_t = 0.0012$ and a median value of $\gamma_p = 0.17$ were chosen. Equations 2 and 3 thus reduce to $\bar{r} = 0.0033\bar{R}$ for total loss and $\bar{r} = 0.4808(\bar{R}/N)$ for percentage loss. Next, the number of transects per stratum can be calculated using Eq 1, assuming the total cost, C, has been specified.

Alternatively, if a given level of precision is specified, n can be calculated by using the variance formulas of the respective estimators. Necessary cost then follows from Eq 1. For example, substitution of estimates of the variance components involved in the formula for the variance of total loss leads to

$$\frac{\text{var}}{t^2} \approx \frac{9 \times 10^7}{n\bar{R}^2} + \frac{5}{n\bar{r}}, \tag{4}$$

and by further substituting a value of $\bar{R} = 850$ and $t = 5$ for the example field in Fig. 1, Eq 4 reduces to var $= 3150/n$, or $n = 3150/\text{var}$.

If the percentage loss is of interest, we can similarly substitute estimates of the variance components involved in the estimator of the variance of the percentage loss estimator to solve for n and hence determine cost. However, for total loss, constant values of these variance components were used to arrive at Eq 4. Such is not the case for percentage loss because the data indicate that as the percentage loss increases, the magnitude of the variance components tends to decrease.

Determination of n depends upon the basic relationship

$$n = \frac{(\alpha_1 S_{bp}{}^2 + \alpha_2 S_{wp}{}^2)t^2}{\text{var}} \tag{5}$$

where

t = number of strata,
var = specified variance,
$\alpha_1 = 1/D^2\bar{R}^2$,
$\alpha_2 = 1/D^2\bar{R}N\bar{r}$, and
D = average sprout density in protected plots.

Values for $S_{bp}{}^2$ and $S_{wp}{}^2$ for a given percentage loss were determined by fitting a straight line to the data points ($S_{bp}{}^2$, percentage loss) and ($S_{wp}{}^2$, percentage loss), respectively. To produce Table 2, values of $D = 50$, $N = 290$, $\bar{R} = 850$, and $\bar{r} = 2$ were used. Necessary costs C for a given variance follows from Eq 1.

Large-Scale Survey Design

For ease of computation, it shall be assumed that all fields are of equal size. It is also assumed that the contribution of within-field variation to the variance of the overall

statewide estimate is negligible. Given this framework, and the simple cost function

$$C = c_0 + c_1 \sum_{h=1}^{t} n_h \tag{6}$$

where

t = number of strata,
n_h = number of sampled fields in h^{th} stratum,
c_0 = overhead cost (essentially represents the total time necessary to travel to all the counties), and
c_1 = cost per sampled field (includes the time necessary to travel between fields in a stratum, as well as the time necessary to select sample points, set out exclosures, and take the data when the susceptibility period is over),

it can be shown (Ref 7, pp. 96–99) that the essential relationship is

$$\frac{n_h}{n} = \frac{N_h S_h}{N_h S_h} \tag{7}$$

where n is the total number of fields to be sampled, t is the number of strata, $S_h{}^2$ is the variance in percentage loss among fields in the hth stratum, and N_h is the number of acres of rice in the hth stratum. If the cost is fixed, n can be determined from Eq 6, and the allocation to strata then follows from Eq 7, assuming estimates of S_h and N_h are available. If precision is specified, then n can be determined from the simple expression

$$n = \Sigma W_h S_h{}^2/\text{var} \tag{8}$$

where

$W_h = N_h/\Sigma N_h$, and
var = specified level of precision.

Equation 8 assumes proportional allocation of fields to strata and a negligible within-field variance component. If, in addition, it is assumed that the within-stratum variances $S_h{}^2$ are equal to, say $S_w{}^2$, then Eq 8 reduces to

$$n = S_w{}^2/\text{var} \tag{9}$$

The number of fields per stratum again follows from Eq 7.

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Mark E. Tobin[1] *and Richard W. DeHaven*[2]

Laboratory Methods for Evaluating Bird Repellents Applied to Ripening Fruits

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ABSTRACT: Tests with caged wild birds for evaluating repellents applied to ripening fruits to reduce bird damage should include (1) sufficient time before testing for the birds to become acclimated to captivity, the test surroundings, and the test procedures; (2) environmental control to reduce stress and variation of results; (3) sufficient length of testing time to identify changes in performance which may occur with time; (4) techniques for measuring variation among individual fruits in size, color, and stage of maturity; (5) evaluation techniques which are appropriate for achieving the objectives of the test; (6) repellency criteria, which will permit a valid comparison of test birds; and (7) treatment concentrations that are at levels far enough apart to minimize overlap. Some general guidelines for complying with these recommendations are given. In addition, an actual test design recently used for comparing the repellency of methiocarb-treated grapes among starlings (*Sturnus vulgaris*), house finches (*Carpodacus mexicanus*), and American robins (*Turdus migratorius*) is described and evaluated.

KEY WORDS: vertebrate pest control, bird repellents, aversive conditioning, methiocarb, ripening fruits, test methods

Continued interest in the use of repellents for protecting agricultural crops from bird damage necessitates the development of standard procedures for screening and testing candidate repellent chemicals. Bird repellents have historically been used for protecting seeds and seedlings, but they are increasingly being used for protecting maturing fruits [*1,2*]. Although some standard laboratory testing methods have been described for seeds [*3*], little has been published on repellent test methods for protecting ripening fruits.

[1] Biologist, Wildlife and Fisheries Biology, University of California, Davis, Calif. 95616.
[2] Wildlife biologist, U.S. Fish and Wildlife Service, Dixon, Calif. 95620.

This paper discusses some general considerations for conducting laboratory tests of the repellency to caged, wild birds of chemicals applied to ripening fruits and describes an actual test design recently used for this purpose.

General Test Design Considerations

Acclimation of Test Birds

For a repellent to reduce bird damage effectively, it must cause a change in behavior which will result in a significant decrease in the birds' utilization of that crop for food [*4*]. Appraising the efficacy of a repellent therefore entails measuring changes in bird behavior either directly by observation or indirectly by measurement of food consumption. It is important that birds used for testing are healthy and are not subjected to stresses that will inadvertently affect their behavior. Recently captured wild birds are often easily agitated and should be held in captivity in communal cages to allow cage conditioning for at least two weeks prior to actual testing [*3*]. If the birds are to be transferred from communal holding cages to novel test surroundings (for example, individual test cages), additional time should be allowed for acclimation to the novel surroundings. Weight can be used as an indicator of progress in cage conditioning. The test birds can be weighed at periodic intervals and actual testing begun only when all the test birds have either stabilized or started to increase their weights since the previous weighing. After the birds are acclimated to the cages, it may be desirable to subject them to all or part of the test protocol if those procedures are likely to cause increased stress when first presented. Also, the investigator should try to ensure that all the birds used for testing have similar pretest experience with the test food.

Test Surroundings

Repellency tests should be conducted in isolated areas free from disturbances. For comparative testing, indoor areas with controlled temperatures and photoperiod are preferable because the possible influence of weather on test results is eliminated. If the test birds are individually caged, the researcher must decide whether to visually isolate them from each other to minimize possible social interactions, since the sight of a conspecific may influence both feeding behavior [*5–7*] and aversive conditioning [*8–11*].

Free-Choice Versus No-Choice Test

Two basic test designs for repellency testing are no-choice feeding trials, in which the birds either eat the food treated with the repellent or go hungry [*3*], the free-choice feeding trials, in which the birds have one or more alternative choices besides the food treated with the repellent [*12*]. The no-choice

test could also be termed the "forced eating" method as opposed to the "preference eating" method of free-choice tests [*13*]. Repellents are likely to be less effective in a no-choice test because the birds are more highly motivated to eat the food treated with the repellent. When free-choice procedures are used, the alternative food should always be specified in the results because repellency may vary depending on which alternative food is available. With either of the two basic test designs, the hunger state of the test birds can influence the effectiveness of a repellent [*13,14*] and should therefore be controlled as much as possible. An effort should also be made to ensure that all of the foods involved in any test are free of pesticides and introduced chemicals other than the repellent being evaluated.

Length of Test

Chemical repellents may produce either a direct aversion to some physical aspect of the food (for example, odor, taste, or color) or a conditioned aversion resulting from an association of some physical aspect of the food with an unpleasant aftereffect (such as illness) [*14*]. Because birds must first experience the harmful consequences of the latter type of repellent before becoming conditioned, repellents which cause conditioned aversions are generally not effective when first encountered. Tests which evaluate such conditioned aversions must therefore measure the birds' performance over a period of time that encompasses this learning process. Also, potential changes in behavior and consumption which may occur during repeated exposures may be important.

Fruit Maturity

Ripening fruits present special problems for repellency testing. The individual fruits (for example, the berries of grapes) may be highly variable in size, color, and stage of maturity, all of which may influence a bird's consumption. Care should thus be taken to ensure that all the berries are as much alike in size, appearance, and stage of maturity as possible. A measure of maturity can often be made by sampling the fruit and using a hand-held refractometer to determine the amount of total dissolved solids (TDS), which provides an estimate of the sugar level in the sample.

Damage Assessment

Bird damage to the fruit can be quantified either by counting the number of individual fruits damaged or by measuring the total weight of fruit taken during feeding. Counting the number of damaged fruits may not accurately

reflect consumption if the fruits are not wholly consumed or if they are not all damaged to the same extent. Some birds may only peck at the skin of the fruit and consume very little of the pulp. The repellent itself may also modify feeding behavior so that birds increase their pecking of treated fruit while decreasing their overall consumption. Therefore, the number of damaged fruits might not be related to actual consumption, and weight loss of the fruit might be a better indicator of repellency. However, if pecking makes the fruits unmarketable, then an evaluation of the number or incidence of pecked fruits may still be appropriate.

Repellency Criteria

Repellency is usually evaluated by comparing each bird's consumption of a repellent-treated food to that of an untreated food or an alternate food. In a free-choice test, both foods are offered simultaneously, and preference for the treated food may be expressed as a proportion of the total food consumption [*15,16*]. In a no-choice test, the alternate or untreated food is usually offered first. The treated food is then offered at a later time and the preference for the treated food expressed as a proportion of consumption of the untreated food [*3,15*].

If an R_{50} (the level or concentration which theoretically repels 50% of the bird population) is desired, then each test bird must be categorized as either repelled or not repelled. One common no-choice method for evaluating seed repellents and estimating R_{50}s is to first offer each bird 25 untreated seeds and to accept for testing only those birds that consume them all. Each bird is then offered 25 seeds treated with the repellent. If 13 or more seeds are uneaten or undamaged, or both, at the end of the test, that individual is considered repelled [*3,17*]. A similar protocol can be applied to tests with ripening fruits.

However, when among-species comparisons are to be made or when variation in food consumption within species is large, a different repellency criterion may be needed. A reduction in consumption of twelve berries would be relatively twice as great for a bird which normally consumes only half as much as another bird. For example, American robins (*Turdus migratorius*) and starlings (*Sturnus vulgaris*) can consume more than three times as many grapes as small birds such as house finches (*Carpodacus mexicanus*) (Tobin, unpublished data). Thus, a comparison among these species of R_{50}s, which were determined with the criterion of the same absolute reduction in the number of grapes consumed, would be misleading. A better comparison might be made by measuring each bird's average consumption of untreated food and then calculating a repellency criterion for each test bird based on its own percentage reduction in consumption. The same percentage reduction in consumption could be applied to each of the species.

Treatment Levels

The quantity of repellent ingested by test birds is a function of the evenness of the application both over each individual fruit's surface and among the individual fruits. Differences in fruit size, position, orientation, and exposure may cause variation in treatment rates. If such variation is large, two or more supposedly different treatment concentrations may actually deposit the same level of chemical on the fruit ([*12*], Tobin and DeHaven, unpublished data). Therefore, to ensure that birds in different treatment groups are actually exposed to different levels of the repellent, treatment concentrations should be at levels far enough apart to minimize any overlap. This should be verified by residue analysis whenever possible.

Data Analysis

The analysis of dose-response data and calculation of R_{50}s can be a complex and tedious task, and several procedures have been used previously for the estimation of R_{50}s [*18–20*]. Although these methods have long been used, particularly when the numbers of test animals or treatment levels are limited, the trend today is toward the use of computers and probit analysis [*21–23*]. The only data required are the number of animals tested at each treatment level, the number of animals repelled at each treatment level, and the actual treatment levels used. Differences in consumption or damage among treatment groups, test periods, and species can also be compared by using an appropriate analysis of variance.

Example of a Test Design

Objectives

Cage tests were conducted to determine the comparative repellency of grapes treated with methiocarb [3,5-dimethyl-4-(methylthio)phenyl methylcarbamate (Mesurol[3])], to house finches, starlings, and American robins—three common bird species found in California vineyards. The objectives were to estimate the R_{50} of methiocarb applied to wine grapes for each of the three species and to examine changes in repellency resulting from repeated exposure to methiocarb-treated grapes.

Procedures

The study was conducted at the Dixon, Calif., Field Station of the Denver Wildlife Research Center. House finches and starlings were captured with

[3]Use of trade names does not imply government endorsement of commercial products.

portable decoy traps in the Napa and Sonoma Valleys of California, and American robins were captured with mist nets near Stockton, Calif., and Bend, Ore. The species were maintained separately in communal, outdoor 4.9- by 2.5- by 2.1-m aviaries. All the birds were conditioned in their communal cages for a minimum of three weeks with maintenance food and water provided *ad libitum*. The maintenance food of the house finches was a seed mixture made up of millet, sorghum, sudan grass, watergrass, and sunflower seed; grit was also provided. The starlings and American robins were maintained on poultry pellets (Albers Eggmaker 20). Bunches of Zinfandel wine grapes were offered periodically to the birds in their communal cages.

The repellency testing was done by using individual birds placed in visually isolated individual outdoor 61- by 46- by 46-cm wire cages which had fiberboard sides, metal roofs, and a removable wire-mesh screen on the floor to catch food spillage. Each cage contained an external waterer and an attached plastic cup containing the same food as was provided in the communal cages. The repellency tests were conducted from November 1980 through April 1981; one species was tested at a time.

The birds that appeared healthy were weighed, placed into an individual cage, and weighed again at the end of each consecutive four-day interval. Pretesting commenced when all of the birds had either maintained or increased their weights since the previous weighing. All of the birds initially lost weight after they were placed in the individual cages; usually about two weeks were required to meet the weight criterion. Previously, a period of seven days has been recommended in the testing of acute avicides (ASTM Test for Efficacy of Acute Avicides [E 551-75]). Conducting the tests outdoors may have extended the cage-conditioning period in our test.

One wine grape variety (Zinfandel) was used for all the tests. Individual bunches of grapes were picked from vineyards on the University of California, Davis, campus during September 1980 and frozen the same day. Bunches were then thawed one day before each test. This permitted the tests to be conducted over extended periods of time and yet have a single source of test grapes.

To reduce variability in sugar content among the test bunches, we estimated the amount of TDS for each bunch by using a hand-held refractometer. One grape berry was picked from the middle section of the bunch, its liquid contents squeezed onto the lens of the refractometer, and a reading in degrees Brix recorded. Only those bunches with refractometer readings $\geq 18°$ were used in the tests.

Repellency tests were no-choice tests involving a series of daily 5-h feeding trials conducted over 13 consecutive days. During each daily trial (from 2 to 7 h after sunrise), each bird's maintenance food was removed from the cage, and one bunch of grapes was suspended above the perch at the center of the cage. At the end of the trial, the grape bunch was removed, the maintenance food was returned, and the number of grapes pecked or removed was

counted. The first three feeding trials were designed to accustom the birds to the testing procedures; the actual test data were collected during the last ten feeding trials. The same procedures were followed for all 13 feeding trials except that untreated grape bunches were offered during the first eight feeding trials (three pretest and five pretreatment feeding trials) and treated grape bunches were offered during the last five feeding trials (five treatment feeding trials). Five or six treatment groups (including a zero or control group) were tested for each species with 16 birds randomly assigned to each group.

Actual treatment of individual bunches took place 1 to 2 h before each feeding trial and was accomplished by immersing each bunch for 3 s in a solution containing the appropriate amount of methiocarb (using Mesurol 75% wettable powder) in 2.0 L of distilled water. The bunches were then hung in a well-ventilated indoor area to dry.

An R_{50} for each species was calculated for each of the five days the birds were exposed to treated grapes. First, the average number of grapes damaged per 5-h pretreatment feeding trial was calculated for each individual bird. Repellency on each treatment day was then determined by comparing the number of treated grapes damaged to the pretreatment average. A bird was considered repelled if the damage was no more than 67% of the pretreatment average. In addition, if the number of grapes damaged by a bird on a treatment day was greater than the number damaged during any one of the pretreatment trials, then the bird was not considered repelled. We calculated the R_{50}s using probit analysis [*23*]. At the end of the repellency test, the birds were returned to their communal cages and provided with maintenance foods. Any illness, unusual behavior, or mortality during the repellency test or the following seven days was recorded.

Suggested Improvements for This Test

Overall, these procedures gave good results for the house finches and American robins, but the results were highly variable for the starlings. Residue analyses completed after the tests revealed that some of the closely spaced treatment concentrations actually resulted in the same or similar residue levels on the grape bunches. This source of variation could have been reduced if the treatment levels had been spaced farther apart. Another problem was that the tests were conducted outdoors during the winter season when daily variations in temperature, wind, and precipitation may have influenced the amount of daily feeding. This situation could have been avoided by conducting the tests either indoors or during another season when weather conditions were less variable. A final problem was the high mortality that occurred among house finches that did not eat grapes, including birds in the zero treatment group. This problem may have been alleviated by reducing

the stress on the birds, either by conducting the test indoors or during a more moderate season or by shortening the length of the feeding trials.

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Jon F. Heisterberg[1] *and David L. Otis*[2]

A Changeover Test Design to Compare the Relative Efficacies of Bird Repellent Seed Corn Treatments

REFERENCE: Heisterberg, J. F. and Otis, D. L., "**A Changeover Test Design to Compare the Relative Efficacies of Bird Repellent Seed Corn Treatments,**" *Vertebrate Pest Control and Management Materials: Fourth Symposium, ASTM STP 817*, D. E. Kaukeinen, Ed., American Society for Testing and Materials, Philadelphia, 1983, pp. 98–109.

ABSTRACT: A changeover test design is described in which treated seed corn was planted and the emerged sprouts were unearthed and offered in different treatment sequences on consecutive days to groups of caged birds. This design exposes each test group of birds to each seed treatment (including untreated) consecutively over time and offers a way to identify and separate any possible residual effects carried over from direct treatment effects. This design eliminates the need for birds to differentiate among treatments offered simultaneously, thus avoiding testing problems associated with the inability to differentiate between treatment areas. The test design corrects some deficiencies of previous cage and field evaluations of chemical seed treatments and has a high degree of statistical precision. The results of one evaluation with three chemical seed treatment repellents indicated that difficulties in assessing bird damage caused by confounding factors, such as uneven germination, insect and disease damage to sprouts, weather-related sprout mortality, and treatment phytotoxicity, can be eliminated by using this test design.

KEY WORDS: vertebrate pest control, repellent, test design, test methods, cage testing, efficacy tests, bird damage, corn sprout damage, seed damage, Mesurol, Curb, Borderland Black, control devices

Chemical seed corn repellents are one of the most frequently used means of reducing bird damage to sprouting corn [*1*]. Recent quantitative surveys of sprout losses to birds in Kentucky and Tennessee have shown, however, that some of the more widely used federally registered seed treatments do not always give satisfactory results (Heisterberg, unpublished studies). These

[1]Research Biologist, U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Kentucky Research Station, Bowling Green, Ky. 42101.

[2]Statistician, U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Denver Federal Center, Denver, Colo. 80225.

surveys prompted us to compare the efficacies of some of these seed corn treatments.

Because of the problems associated with testing of chemical repellents in commercial fields, we initially chose a cage test method similar to that described by Linehan [*2*]. Linehan's testing procedures called for the use of small plots of planted and emerged, treated and untreated, seed corn offered to a group of caged birds at the same time. This method assumes that birds can distinguish between treatments offered simultaneously in a relatively small, confined area and does not consider findings of other investigators who have observed that birds have difficulty distinguishing among small plots [*3–5*]. To eliminate this potential problem, we chose a changeover test design [*6*]. Such designs have been described and widely used in dairy feeding trials over the past 40 years [*7–9*], but their use elsewhere has apparently been limited. To use this design for our situation, groups of birds were exposed to one treatment at a time, with each group of birds consecutively receiving all the treatments. Because of the chemical nature of the treatments, it was thought that there existed some potential for residual effects from a treatment given in the preceding period. Therefore, a particular changeover design was chosen that allows estimation of and adjustment for these potential residual effects.

Initially, damage was assessed following Linehan's method of placing caged birds (common grackles, *Quiscalus quiscula*) over both bird-protected and unprotected emerging corn sprouts and then determining the damage [*2*]. However, several problems arose (Heisterberg, unpublished studies). Foremost among these was that caged birds were not consistent in pulling or digging planted corn sprouts, and they often trampled and clipped off sprouts without unearthing the corn seed. Such mechanical damage was sometimes severe enough to result in the sprout not emerging even though the seed had been untouched. We also encountered problems in both bird-protected and unprotected plots with uneven germination of seeds caused by insects, weather conditions, and possible phytotoxic effects of seed treatments. Differentiating between insect damage (either to the seed or to the subsurface cotyledon portion of the sprout), bird damage, and phytotoxicity was often difficult or impossible. To eliminate these problems, we unearthed treated, emerged corn sprouts and offered them to birds. This paper presents the results of a comparison of the bird repellent efficacies of three seed corn treatments using unearthed sprouts and the changeover test design.

Methods

During July 1981, the bird repellent efficacies of Mesurol 50% Hopper-Box Treater[3] (Mobay Chemical Corp., Kansas City, Mo.), Borderland Black

[3]The use of trade names does not imply endorsement by the federal government.

(Borderland Products, Inc., Buffalo, N.Y.), and Curb (Sphere Laboratories Ltd., London) were evaluated against an untreated control. The recommended label application rates of 0.5 kg per 100 kg seed corn for Mesurol (0.25% methiocarb active ingredient/weight of seed), 0.9 kg per 100 kg seed corn for Borderland Black (0.17% methiocarb active ingredient/weight of seed), and 5 kg per 100 kg seed corn for Curb (predominantly aluminum ammonium sulfate) were used. Mesurol and Borderland Black are registered in the United States as bird repellent seed corn treatments [*10*], and Curb is used in Europe as a bird repellent seed corn treatment [*11*]. Pioneer 3147 hybrid seed corn (Pioneer Hi-Bred International, Inc., Des Moines, Iowa), pretreated with 0.06% captan and 0.0015% malathion (standard seed corn fungicide and insecticide treatments), was used in the study. Seed corn was surface-treated by adding the specified amount of bird repellent to 1 kg of seed corn in a 2-L jar. During planting it was necessary to shake the jar frequently to keep the chemical adhered to the seed.

Common grackles, trapped in the early summer and cage acclimated for a minimum of two weeks, were used in the study because they are reportedly the species that cause much of the corn sprout damage in the southeastern United States [*1*]. All the birds were given poultry pellets and water *ad libitum* before and during all testing. Two birds that escaped during the interval between test periods were replaced from a pool of birds subjected to the same sequence of treatments as the birds they replaced.

We followed the design described by Federer [*6*], involving three, 4-by-4 orthogonal Latin squares. A total of 36 grackles were allotted to one of three equal-sized groups to maximize among-group heterogeneity while minimizing within-group heterogeneity. Age and sex were used as the differentiating criteria; groups consisted of twelve after-hatching-year males, twelve after-hatching-year females, and twelve hatching-year birds of unknown sex. Birds within each group were randomly assigned to one of four units (grackle units) of three birds each. A 4-by-4 Latin square block was randomly assigned to each of the three groups and, in turn, each grackle unit within a group was randomly assigned to one of the treatment sequences (Table 1).

The seed corn used was planted six to eight days before the beginning of the first test period. Sprouts averaged 3 to 10 cm tall when unearthed and offered to the birds. The test birds were preconditioned to eating unearthed sprouts by offering each grackle unit an average of 100 untreated sprouts each day beginning three days before the first test period. During each of four, 3-h test periods, each separated by a 21-h rest period, each grackle unit received 120 sprouts of the designated treatment. These sprouts were unearthed in the afternoon, placed in a plastic bag and refrigerated overnight, and then offered to the birds from 8:30 to 11:30 A.M. the following morning. All unearthed sprouts offered to birds had roots, seed, stem, and leaves intact, but most of the soil was shaken loose from the roots. The unearthed sprouts were placed 6 cm apart on either side of a 5-cm wide wood template

TABLE 1—*Treatment sequence (A = Curb, B = untreated, C = Borderland Black, D = Mesurol) and number of unearthed corn sprouts eaten by grackle units in three Latin square blocks over 3-h test periods.*

	Three After-Hatching-Year Females per Cage Unit					Three After-Hatching-Year Males per Cage Unit					Three Hatching-Year Birds (Sex Unknown) per Cage Unit					
Test Period (Date)	Unit 1	Unit 2	Unit 3	Unit 4	Total	Unit 5	Unit 6	Unit 7	Unit 8	Total	Unit 9	Unit 10	Unit 11	Unit 12	Total	Grand Totals
I (9 July)	A = 104	B = 91	C = 33	D = 43	271	A = 87	B = 83	C = 59	D = 21	250	A = 64	B = 100	C = 18	D = 37	219	740
II (10 July)	B = 89	A = 95	D = 11	C = 65	260	D = 26	C = 51	B = 79	A = 3	159	C = 31	D = 19	A = 30	B = 28	108	527
III (11 July)	C = 30	D = 30	A = 1	B = 23	84	B = 20	A = 35	D = 20	C = 3	78	D = 26	C = 38	B = 14	A = 96	174	336
IV (12 July)	D = 24	C = 20	B = 11	A = 96	151	C = 9	D = 29	A = 5	B = 4	47	B = 42	A = 33	D = 15	C = 68	158	356
Totals	247	236	56	227	766	142	198	163	31	534	163	190	77	229	659	1959

and were offered to each grackle unit in a 1.5- by 3- by 1.5-m-high cage (Fig. 1). All twelve cages were located outdoors and occupied a 3- by 18-m area (Fig. 2).

Damage to corn sprouts was assessed after a 3-h exposure to each grackle unit. A sprout was recorded as damaged when the seed had been partially pulled apart or totally eaten. All the damaged or undamaged sprouts could normally be accounted for during the assessment, however, in the few instances in which they could not, the missing sprouts were considered eaten by birds. The total number of damaged sprouts in each cage was used in analysis of variance calculations, as outlined for the changeover design [*6*]. Duncan's new multiple range test and Tukey's *w* procedure [*12*] were used to identify differences among treatments.

Results and Discussion

There were no problems with acceptance of unearthed corn sprouts by grackles. During preconditioning trials, the birds readily ate untreated kernels from freshly unearthed sprouts and appeared to prefer these over the alternate poultry pellets. Sprouts damaged by the birds were easily identified. The kernel was usually picked apart, with the remains still left attached to the stem and roots. Most of the sprouts eaten were consumed during the first hour of each test period since the sprouts dehydrated rapidly and birds found such sprouts less appealing.

The number of corn sprouts eaten by each grackle unit is given in Table 1. Analysis of variance calculations yielded significant differences ($P < 0.05$) among test periods, grackle units within Latin square blocks, test period by block interaction, and both direct and residual treatment effects (Table 2). Differences among test periods may have been a result of birds developing an aversion to feeding on sprouts as the test progressed (Table 1). A similar pattern of decreasing sprout consumption over time was observed when using this test design to compare efficacies of another group of bird repellents (Heisterberg, unpublished studies). Differences among grackle units within blocks reveal the significant variation in response among cages within the same age/sex class. The period by block interaction indicates the relationship among the blocks in total sprouts damaged changed over time. Because of the design used, the differences among test periods, units within blocks, test period by block interaction, and residual treatment effects could be removed from the experimental error, thereby resulting in a more efficient and sensitive comparison of the bird repellent efficacies of the seed corn treatments.

Using techniques described by Federer [*6*], we adjusted the treatment means to compensate for the residual treatment effects (Table 3). The adjustments reduced the mean number of damaged sprouts treated with Mesurol and increased the mean number of damaged sprouts for both Curb-treated

FIG. 1—*Using a wooden template for placement and counting of unearthed corn sprouts before offering them to the caged grackles.*

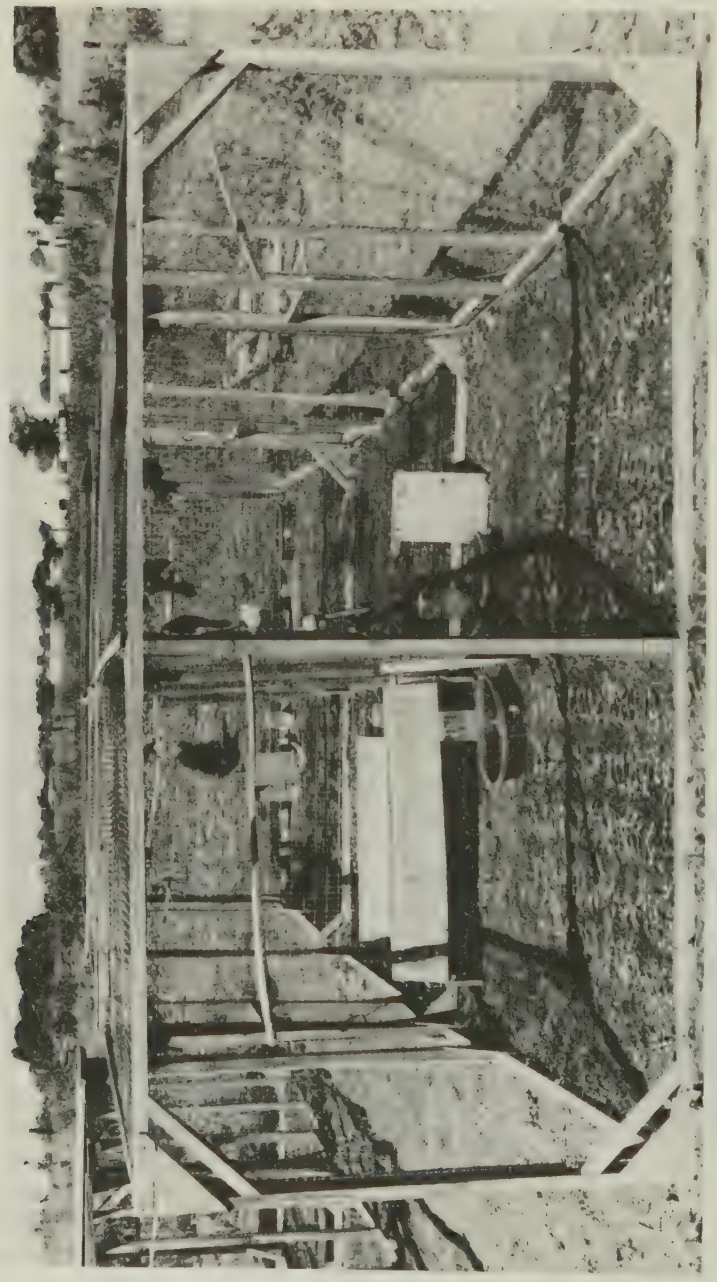

FIG. 2—*End view of the twelve-cage setup used to offer grackles unearthed corn sprouts.*

TABLE 2—*Analysis of variance relating unearthed corn sprouts with different seed corn treatments and consumption by common grackles.*

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F*	*P*
Total	47	44 951.3	...	...	...
Blocks (groups)	2	1 685.4	842.7	2.7	0.091
Test periods, days	3	8 795.1	2 931.7	9.5	<0.005
Grackle units (cages) within blocks	9	13 189.7	1 465.6	4.8	<0.005
Test period × block	6	5 027.1	837.8	2.7	0.042
Treatments (direct and residual)	6	9 782.1	...	...	...
Direct (ignoring residual)	3	6 171.9	...	...	...
Residual (eliminating direct)	3	3 610.2	1 203.4	3.9	0.023
Direct (eliminating residual)	3	8 516.8	2 838.9	9.2	<0.005
Residual (ignoring direct)	3	1 265.3	...	...	...
Error	21	6 471.9	308.2	...	...

TABLE 3—*Mean consumption per test period per grackle unit of unearthed corn sprouts. Any two means not connected by the same line are significantly different (*$P < 0.05$*).*

	Unadjusted		Adjusted for Residual Effects	
Treatment	Mean	Percent Increase Over Mesurol	Mean	Percent Increase Over Mesurol
Mesurol	25.1	...	21.0	...
Borderland Black	35.4	41.0	34.5	64.3
Untreated	48.7	94.0	51.7	146.2
Curb	54.1	115.5	56.0	166.7

and untreated sprouts. The mean number of damaged sprouts was about the same for Borderland Black-treated sprouts, indicating that the positive residual effects from Curb and untreated sprouts were offset by the negative residual effects from Mesurol. Separation of the mean consumption values for different treatments using Duncan's new multiple range test shows the same significant differences between treatments using unadjusted and adjusted means, except for Borderland Black-treated and untreated sprouts (Table 3). By using unadjusted means, the false conclusion would have been drawn that Borderland Black did not protect corn sprouts from bird damage. Differences between the consumption of Mesurol-treated sprouts and the consumption of sprouts treated with other substances were increased by the adjustment for residual effects.

Federer also presented a method for estimating the difference between the residual effects of two treatments. The significance of these differences has

been evaluated by Tukey's *w* procedure, which requires independence of treatment means and an unbiased estimate of experimental error. Because the pair-wise differences between residual effects are probably slightly correlated, and an approximate value for the standard error of an estimated residual effect was used, the true significance levels of the differences may differ slightly from those given in Table 4. However, Table 4 does provide evidence of significant differences between the residual effects of Mesurol and Curb and between Mesurol and no treatment. No differences involving the other two treatments were indicated. To illustrate the meaning of the estimates in Table 4, consider the estimated difference of −28.3 for the Mesurol-treated and untreated sprouts. Roughly speaking, this estimate implies that grackle units which received sprouts treated with Curb or Borderland Black after Mesurol-treated sprouts tended to consume significantly fewer sprouts than units that received Borderland Black-treated or Curb-treated following untreated sprouts. Therefore, from a practical standpoint, Mesurol apparently not only offers the best protection of the three treatments but also offers the greatest residual action, a desirable characteristic of any bird repellent seed treatment.

A final point of interest is that the magnitude of the difference in direct effects between any two treatments is very close to the magnitude of the corresponding difference in residual effects (Tables 3 and 4). Thus, whatever difference exists between the direct effects of two treatments apparently is sustained over the next treatment period.

Changeover designs that account for the presence of residual treatment effects require a larger number of experimental units than simpler changeover designs that assume no residual effects. For example, we could have used a design that requires only eight groups of birds, yet generates nearly as many degrees of freedom for error (18) as the design used (21), had the assumption been made that none of the treatments had a residual effect. To judge whether the increased effort was justified requires an examination of the magnitude of the estimated residual effects. Table 3 showed that adjusting

TABLE 4—*Estimates of the difference between residual effects (sprouts consumed/grackle unit/test period) of pairs of treatments. The significance of the difference was determined by Tukey's w procedure.*

Treatments	Estimate	*P*
Mesurol–Borderland Black	−12.7	>0.10
Mesurol–Curb	−24.1	0.10
Mesurol–untreated	−28.3	0.05
Borderland Black–Curb	−11.4	>0.10
Borderland Black–untreated	−15.6	>0.10
Curb–untreated	−4.2	>0.10

the treatment means for residual effects did not have any real impact on the relative ranking or separation of the treatments. The largest adjustment was made to the treatment mean for Mesurol; this treatment was also involved in both of the comparisons of residual effect differences that tended toward significance (Table 4). Although the increased effort did produce information indicating that Mesurol apparently does have some residual effect, it is probably true that the main objective of the experiment, namely, evaluation of the relative efficacies of the chemical treatments, could have been successfully achieved with a simpler changeover design. However, this evaluation is *a posteriori*, and we would recommend that researchers involved in testing chemical repellents assume that residual effects represent a potential biasing factor, unless data are available that indicate otherwise.

The changeover test design can be used to determine the direct and residual effects of three or more treatments, although, as the number of treatments to be compared increases, so does the required sample size and number of test periods. For example, five treatments require 20 units repeated over five test periods, seven treatments requires 42 units repeated over seven test periods, and so on. With a slight modification of the test design, the direct and residual treatment effects can also be determined for two treatments [*8*].

One possible drawback of the changeover test method is that it is designed and analyzed based on the assumption that the residual effects of a treatment carry over only one test period. If the residual effects of a treatment carry over for more than one period, a longer rest period between test periods may eliminate extended carry-over. Further, there are changeover designs that permit estimation of residual effects that last more than one test period [*13, 14* as cited in Ref *8*]. Our study, however, did not appear to indicate a carry-over of more than one period for any of the seed treatments tested.

We can make several suggestions on how to improve this test design. It may be better to have longer test periods, such as offering birds the same treatment several days in a row. This would allow the birds more time to react to each treatment, resulting in a more realistic test. We also suggest that different cages of birds be visually screened from each other. This is to avoid any possible social interactions among birds in different cages, which could influence feeding behavior [*15*].

Conclusions

The changeover test design has the distinct advantage of having each test unit receive each treatment consecutively over time and offers a way to identify and separate both direct and residual treatment effects. This eliminates the need for birds or other animals to have to learn to differentiate among different treatments offered at the same time. Because each bird unit is exposed to each treatment, the effect of a treatment is evaluated by judging the

response of a bird unit to that treatment in relation to the response of that unit to each of the other treatments. Thus, experimental error due to natural variation in consumption among cages of birds is removed, and more sensitive treatment comparisons are possible. This design allows the investigator to determine how different bird units respond to the same treatment and how the same bird unit responds to different treatments. The design requires a relatively small number of birds for sensitive comparisons of treatments.

Because residual treatment effects of bird repellents can be an important part of a chemical repellent's effectiveness, it is desirable to have a test design that can identify and separate residual effects from direct treatment effects. Although residual effects will not always be present, this test allows for appropriate corrections when such effects are encountered [7].

The use of unearthed sprouts to compare efficacies of different seed corn treatments eliminated many of the confounding environmental conditions imposed by a planted seed corn test and resulted in adequate bird pressure. Damage assessment techniques were straightforward and rapidly accomplished. The efficacies of bird repellent treatments on seeds of crops other than corn (for example, wheat and rice) could also be evaluated by the unearthed sprout method. The basic test design could also be used to evaluate treatment effects of topically applied bird repellents on fruit.

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Mark H. Merson,[1] *Ross E. Byers,*[1] *and Dale E. Kaukeinen*[2]

A Test Protocol for Assessment of Primary Poisoning Hazard to Ground-Feeding Birds from Broadcast Pelletized Rodenticides

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ABSTRACT: A test protocol was developed after the large-pen field study guidelines suggested by the U.S. Environmental Protection Agency to assess potential primary poisoning hazard to birds from broadcast pelletized rodenticides used in orchards. The test employed six pen-reared ring-necked pheasants (*Phasianus colchicus*) confined in each of twelve stationary 0.0047-ha pens. The pens were erected over ground cover similar to that in an adjacent orchard. The bait material was broadcast in the pens at zero (control), one, two, or ten times a base rate. The pheasants were maintained on a grain and seed mixture, which was broadcast into the pens. The advantages of the test protocol and the rationale for some of its features are discussed.

KEY WORDS: vertebrate pest control, hazard assessment, primary poisoning, birds, pheasants, rodenticides

The broadcast application of pelletized rodenticide baits in orchards to control voles (*Microtus* spp.) has proven to be both efficaceous [*1,2*] and less time-consuming and labor intensive than traditional hand placement of bait. When broadcast, the bait pellets are widely dispersed in an under-tree band on approximately two thirds of the orchard floor rather than placed at strategic points in active vole burrows and runways. The distribution of

[1]Research associate and professor of horticulture, respectively, Winchester Fruit Research Laboratory, Virginia Polytechnic Institute and State University, Winchester, Va. 22601.

[2]Technical representative, ICI Americas, Inc., Biological Research Center, Goldsboro, N.C. 27530.

pellets during broadcast application has raised questions concerning contact of ground-feeding birds with the rodenticide and possible primary poisoning hazard. The value of broadcast application in controlling orchard voles, coupled with the possibility of primary poisoning hazard, necessitated the development of a protocol for assessing potential primary poisoning hazard to birds from broadcast pelletized rodenticides should available toxicological data [median lethal dose (LD_{50}), median lethal concentration (LC_{50}), and so on] indicate a need for concern. The following test protocol is an adaptation of the general recommendations by the U.S. Environmental Protection Agency (EPA) concerning large-pen field studies for evaluating hazard to birds [*3*]. Both the pen design and test procedure were developed in consultation with representatives of EPA and U.S. Fish and Wildlife Service.

Materials and Methods

Test Pens

The test pens were stationary and constructed from welded wire dog kennel units (Sears and Roebuck, Inc.). Each test pen was 2.4 by 19.5 by 1.2 m and approximated the minimum surface area (0.005 ha) suggested by the EPA guidelines for large-pen tests. Twelve pens were constructed side by side (Fig. 1). The entire pen complex was surrounded by aluminum flashing sunken 0.15 m into the ground and extending to a height of 0.5 m above the ground. A tractor-mounted chisel plow was used to create a trench for the buried portion of the flashing. To further discourage predators, an electric fence was installed around the pens at a distance of 0.3 m. The electric fence was two-stranded with the lower strand approximately 15 cm above the ground and the upper strand 46 cm above the ground.

Black roofing paper was fastened between adjacent pens to prevent cross-contamination with the toxicant, to reduce interpen disturbances, to act as a windbreak, and to prevent the likelihood of observationally learned food aversions between pens. The back wall of each pen was also covered with roofing paper, and a 1.2- by 2.4-m (4- by 8-ft) sheet of particle board was placed over the end of the pen for shelter. A small door was located at the opposite end of each pen for access. The pens were erected adjacent to an orchard in an area with ground cover similar to that in the orchard. Insufficient space between tree rows prevented placing the pens directly in the orchard.

Test Procedures

Adult, pen-raised pheasants (*Phasianus colchicus*) of uniform age and healthy appearance were introduced to the test pens (six per pen). Before their release into the pens, the birds were weighed and individually marked using wing clip tags. Throughout the experiment, the pheasants were fed a

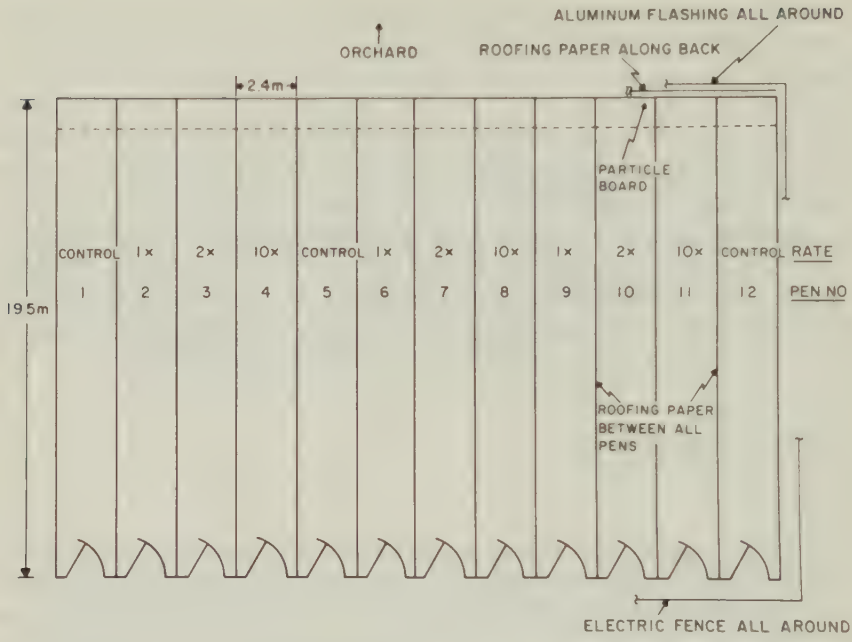
ALUMINUM FLASHING ALL AROUND
ORCHARD
ROOFING PAPER ALONG BACK
2.4m
PARTICLE BOARD
CONTROL 1× 2× 10× CONTROL 1× 2× 10× 1× 2× 10× CONTROL RATE
1 2 3 4 5 6 7 8 9 10 11 12 PEN NO
19.5m
ROOFING PAPER BETWEEN ALL PENS
ELECTRIC FENCE ALL AROUND

FIG. 1—*Schematic diagram of pen complex and treatment assignment to pens.*

grain-seed mixture of sunflower seeds, oats, wheat, barley, and clover/grass seed (all approximately 20% by volume). No hulled or cracked grains were used. The varied particle sizes used in the mixture avoided the possibility of conditioning the pheasants to a certain particle size. The birds had been reared on a commercial poultry crumble.

At the request of the EPA, the birds were fed both before and after treatment on a four-day cycle of feeding three consecutive days and fasting the fourth day. The seed was broadcast into the pens from alternate ends at each feeding at a rate of 100 g of seed per bird per day. A ration of 100 g per day was considered by consulting experts to be adequate for bird maintenance. Each pen had water available *ad libitum*.

The toxic bait was hand broadcast as evenly as practicable in the pens on the fifth day after the birds were introduced. A four-day acclimation period was used to avoid excessive trampling of the ground cover. The day of treatment was preceded by the first day of fasting. The birds were immediately fed after the bait was applied.

The bait was applied at rates of zero (control), one, two, or ten times a base rate, X. The expected use rate of the rodenticide was between the $1X$ and $2X$ rates. Each treatment rate was replicated three times, as was the control. A control pen was on each end of the pen complex (Fig. 1). A center pen was made the third control pen, and the other treatments were assigned so

that no adjacent pens received the same treatment rate. Assigning treatments in this manner roughly distributed pen differences across treatments. Daily weather records were kept throughout the experiment.

The birds were observed daily for three weeks after treatment for mortality or other signs of rodenticide poisoning. At the end of this period the birds were weighed and released into the same pens. The pheasants were then held for another two weeks, after which the survivors were weighed and sacrificed. Birds having died or showing signs of poisoning were necropsied. A representative sample of survivors was also examined for sublethal effects. The size of such a sample would depend on the toxicological characteristics of the material being tested.

Evaluation of Hazard

Mortality in the test birds would, of course, be the most obvious indication of primary poisoning hazard. The occurrence of sublethal effects of rodenticide exposure (such as body weight loss, behavioral changes, gross pathological signs, reversible symptoms of intoxication) is also important information to the investigator in assessing potential hazard. These sublethal effects, while not resulting in death in the relatively sheltered conditions of the test pens, may be of more significance under actual field conditions. The amounts of material available to the pheasants in the low-rate pens may not be sufficient to cause mortality in all the birds in the pen but allows the possibility of sublethal exposure and examination of individual variation in bait ingestion and manifestation of effects.

Residue analyses of tissue from dead birds and survivors should be performed to obtain information as to bait acceptance. In addition, residue data, when viewed in light of mortality, behavioral changes, and gross pathology, can give insight into the significance of contamination which may occur under actual use. If residue analysis should not be possible, bait acceptance could be measured by the use of inert markers [*4,5*].

Frequency data such as mortality can be analyzed using a chi-square test for independence. Continuous variables can be analyzed by analysis of variance. The sample sizes used in development of the protocol were considered adequate but minimal. Detection of subtle differences between formulations would probably necessitate the addition of more pens or replicate experiments.

Discussion

Test Species and Observation Period

The ring-necked pheasant is a species suggested by EPA guidelines for use in large-pen field studies [*3*]. The use of ring-necked pheasants in tests of or-

chard rodenticides could be justified because of this species' common occurrence in many apple growing regions of the United States and because of its economic and recreational importance. Whether the ring-necked pheasant is the most appropriate species for testing or the only species that need be tested depends on factors which have not yet been completely assessed. Among these factors are the relative sensitivity of the pheasant to the rodenticide being tested and method of application. The variable toxicity of chemicals to different avian species and the "indicator species" concept in hazard assessment have been discussed by others [*6, 7*].

Should hazard data on other bird species be desired, the test method could be readily adapted to meet the peculiar requirements of these species. In addition, modifications could be made according to the characteristics of the rodenticide being tested. For example, the observation period could be lengthened or shortened depending upon the rapidity of action of the material.

Test-Related Stressors

Several features of the test method (pens, timing, stocking) were designed to reduce stress and the possibility of injury to test birds. The authors felt this to be an important consideration in the case of the anticoagulant rodenticides since stress has been shown to enhance the effects of such compounds [*8, 9*]. Undue stress or injuries to the test birds resulting from conditions of their captivity or handling procedures could unfairly bias the test results and make repeatability of results from test to test difficult to obtain.

Available information indicated that prevention of harassment of the birds by predators would be an important aspect of preventing possible injury and reducing stress. It was suggested in a letter dated 8 Sept. 1978, from the National Agricultural Chemical Association (NACA) to the EPA that flashing be used around pen edges in large-pen tests to prevent predators from digging under pens. Instances of this had occurred in pen studies conducted by NACA members. In addition, NACA members had observed in their tests that quail would fly wildly against the sides of the pens when predators became visible.

Harassment of the pheasants by domestic dogs was encountered by the authors before installation of the electric fence. The dogs would repeatedly chase the birds the length of the pens with the two end pens being most affected. After installation of the electric fence, the problem subsided after two days. An electric fence could possibly be used with portable test pens [*4*], but the daily movement of the portable pens would necessitate moving the fence also. The authors placed the electric fence close (0.3 m) to the pens to ensure continued contact with the fence if a predator got between the fence and pens.

Because agitation of the birds in the end pens was greatest, the decision

was made to place the control pens on the end of the pen complex to protect the possibly more vulnerable treated birds from any further harassment. While this sacrificed randomized assignment of treatments to pens, it was viewed as a necessary compromise. Investigators doing similar tests and having an area completely secure from predators may wish to completely randomize pen treatment assignment.

The seasonal timing of a test should, ideally, coincide with the intended time of use of the material being tested. In the case of orchard rodenticides, this would be the postharvest dormant period. The problem with performing outdoor tests at this time of the year is the changeable nature of the weather and variability of weather from one year to the next. Weather factors complicate the interpretation of tests done in successive years, since there are associated problems of unequal bait degradation and level of test bird stress. Investigators should use their own discretion when deciding on when to conduct their tests, taking into account local conditions.

Some weather-related problems could be circumvented by using a roof over the test pens, but this approach prevents bait degradation due to normal weathering. The authors viewed this as a major disadvantage and, instead, chose to perform their tests without roofs but at a time when the weather was more predictable and moderate. The majority of the authors' tests were conducted at a time which would be considered early in the intended use period of the rodenticide being tested but when weather conditions were less severe.

The first of a series of three such tests performed in Virginia was done from late November to January. During this time, minimum temperatures below freezing and precipitation in the form of snow, sleet, and freezing rain were encountered. Although no mortality occurred in the control groups, the control birds experienced a 14% loss in initial body weight, indicating that the experimental ration was inadequate under such conditions. Because the authors were concerned about the hardiness of pen-reared birds under severe weather conditions, the subsequent tests were performed in September and October when conditions were more moderate. In these subsequent tests, the surviving birds showed a mean overall weight increase of 19 to 21%, indicating that their food requirements were more than adequately met by the experimental ration. The birds would then not be forced involuntarily to the pelletized bait as a food source.

In a typical maintained orchard situation, and especially with mowed sod ground covers, little existing food for the birds is present. Therefore, when the test pens are set up over orchardlike ground covers, most food for the birds must be supplied exogenously. Broadcasting the feed in the pens, however, probably more closely simulates natural conditions by requiring the birds to search among the pellets on the pen floor for food rather than coming to a feeding station. The birds were not fed one day out of four to avoid an excessive accumulation of grain and seed on the pen floor and to provide a degree of feeding stress to the birds, as would be expected under natural conditions.

The stocking rate of six pheasants per pen did not appear to excessively crowd the birds. There were indications, however, that social conditions within the pens can be an important factor. In one trial the pheasants were stocked at a rate of one male and five females per pen. The males were significantly lighter in body weight than the females and were subject to harassment by the females. As a result, nine of the twelve males died during the experiment, including two from the control pens. The dead males showed evidence of having been pecked. In a subsequent trial, all female pheasants of similar age were used (although males may have worked just as well), and no significant intrapen strife was observed. In stocking the pens, efforts should be made to select test birds in such a way as to avoid social stresses which are an artifact of the confinement situation.

Handling procedures can be a significant factor in the test protocol and could influence the results obtained. In the second year of testing the pheasants were recaptured from the pens 23 days after treatment, weighed, and then returned to the pens for further observation. Four birds (three of which were untreated controls) died subsequent to the weighing. These deaths were suspected of being related to stress or injury resulting from the weighing procedure, as all previous mortality had occurred by 15 days after treatment. Recovering the birds from large pens can be taxing on them because the pheasants will run or fly against the pen sides when pursued. Again, because of the enhanced toxicity of the anticoagulants to animals during stress [*8, 9*], the investigator should try to keep handling of the birds to a minimum and handle them in the gentlest manner possible.

Conclusion

This test protocol has proven satisfactory in assessing the potential primary poisoning hazard from pelletized rodenticides to pheasants in the orchard environment. The real utility of the method is in obtaining a relative index of potential primary poisoning hazard between baits and labeled application rates and not as a substitute for a full-scale field test under actual use conditions. A series of tests run in the same location under similar conditions, such as over successive years, can be useful in comparing different toxicants or different formulations of the same toxicant. During protocol development, the mortality for the 2*X* and 10*X* rates of an anticoagulant rodenticide was 33 and 100%, respectively. Modifications in the formulation and concentration of active ingredient were made and, in the third and final test of the same toxicant, the mortality was reduced to 6% at 2*X* and to 39% at 10*X*.

The general testing method would be readily adaptable for use with other bird species and toxic materials if initial laboratory toxicological tests should indicate the need for large-pen studies. Investigators desiring to conduct studies for the purpose of satisfying registration requirements for specific compounds and formulations should develop a specialized protocol in con-

sultation with the appropriate regulatory authorities. The major advantages of this method are that (1) the large pens meet the dimensions suggested by the EPA for such tests, (2) the stationary pens allow adequate protection of the test birds from predators, and (3) the diet and its method of presentation ensure exposure of the birds to the toxicant without the chance for preconditioning the birds to consume the bait particles.

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Stephen A. Shumake,[1] *Stanley E. Gaddis,*[1] *and Melvyn V. Garrison*[1]

Development of a Preferred Bait for Quelea Control

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ABSTRACT: Three factors that could influence bait acceptance and palatability (seed size, hardness, and color) were evaluated on small groups of African red-billed quelea (*Quelea quelea*). The birds were first adapted to feeding on mixed grain from automatic two-choice preference testing devices that alternated positions of the standard and test baits every 5 min. Before testing, the birds were exposed to all levels or formulations of bait for 6 h per day for five days. Preference test data were obtained in four 6-h sessions by using relative consumption (percent preference for test bait) as the dependent measure. Particle sizes tested ranged from 0.3 to 1.8 mg/granule; 6.0 mg hulled proso millet was the standard. All quelea preferred standard whole seed to granules of millet. Ground millet flour, bentonite, and different moisture levels (8, 10, 14, or 16%) were used to achieve different bait pellet textures. This variable had an effect on preference at the lowest (8%) moisture level, indicating that soft, dry pellets are significantly rejected by quelea. Color preference data indicated that red or yellow-green colored millet seed was rejected when compared with the standard yellow millet; however, these effects were not within acceptable levels of statistical significance ($P < 0.07$). When light levels were reduced and the color-preference test replicated, significant ($P < 0.01$) preference changes were noted, suggesting that feeding behavior tended to be random with the color stimuli made less visible.

KEY WORDS: bait, bird, color, texture, particle size, preference test, *Quelea quelea*, Africa, millet, vertebrate pest control

African red-billed quelea (*Quelea quelea*) cause extensive damage to agricultural crops (millet, sorghum, rice, and wheat) throughout many areas of Africa [*1*]. Despite wide-scale aerial spraying of nest and roost sites with parathion and fenthion, control is often ineffective and costly. Most of our recent research [*2,3*] on the quelea problem has involved the development of repel-

[1]Research psychologist, biological technician, and research physiologist, respectively, U.S. Fish and Wildlife Service, Denver, Colo. 80225.

lent chemicals for direct application to maturing grain. The success of this control method, of course, depends on the availability of alternative noncrop foods, such as weed and grass seeds, during the crop growing season. In those situations in which no alternative foods are available to quelea and other depredating species, avicides may have to be used instead of repellents to ensure crop protection. A means of chemically affecting large segments of a depredating flock would therefore be necessary and could involve baiting programs in agricultural areas.

The development of a grain or pelleted bait material depends on a standardized method of preference testing. The rationale for developing improved baits includes (*a*) increasing the number of affected birds, (*b*) increasing acceptance of avicide or other control chemical, (*c*) reducing the costs of application, and (*d*) improving the data base for quelea management.

The bait preference tests in our study were designed to yield information on the usefulness of an automatic two-choice preference tester; we also wanted to evaluate several factors that could affect bait acceptance in quelea: particle size, pellet hardness, and bait color. Particle size was tested to determine if quelea prefer small seeds [*4–6*], as reported from field research. Bait pellet hardness was assessed to determine if it would improve or interfere with preference for or acceptance of a bait. Bait color was studied to determine whether the birds show any natural tendency to eat seed material of a specific color class.

Materials and Methods

Bait Particle Size Evaluation

Three particle sizes [0.1 to 0.3 mg (20 to 28 mesh), 0.4 to 0.8 mg (16 to 20 mesh), and 1.6 to 2.0 mg (14 to 16 mesh)] were tested with whole hulled proso (that is, the dark-colored variety) millet (6.0 mg/seed) as the standard. Millet seed was ground using a Thomas-Wiley[2] mill (Model ED-5) and subsequently sifted into the three particle-size ranges with a series of standard sieves (Nos. 14, 16, 20, 28, and 35) and a sieve-shaker machine.

Fifteen male quelea were acclimated to double (38- by 25- by 17.8-cm) cages, each divided with 1.3-cm wire mesh so that the birds could be individually housed but allowed to hear and see one other bird [*2*]. After two weeks of acclimation, automatic preference test devices (Fig. 1) were placed in the cages. These devices reverse the left-right food positions every 5 min to reduce position bias.

Food cup rotation was controlled by switching circuits and could be programmed for intervals of 5, 15, 30, and 60 min. Light intensity in the cages was controlled by a rheostat connected to individual 7 W (110 VAC) lamps.

[2]Reference to trade names does not imply endorsement of commercial products by the federal government.

FIG. 1—*Male quelea tested for bait preference. The two food cups rotate left and right positions every 5 min during testing, and light levels in the cages are controlled by a rheostat. The light fixture is at the top center of the photo.*

The birds were first adapted to feeding from the food cups without the rotation motor being in operation for five days. During this period, quelea were offered an equally proportioned mixture of the three granule sizes with 25 g in each of the two available cups. The devices were successively programmed to rotate every 60, 30, 15, and 5 min over the following seven days so that the birds were gradually habituated to the movement of the feed cups. All the birds were then given two consecutive uniformity tests (6 h/day for two days): one with whole hulled millet and the second with mixed millet granules. All the tests were conducted ½ h after the beginning of the light portion of a 12:12 light-to-dark cycle, and room temperature was maintained at 25°C ± 2°C. The birds were allowed free access to their normal mixed grain diet for 3 h per day after each test session. Finally, the 15 birds were randomly assigned to three test groups ($n = 5$ birds/group) to receive either 20 to 28 mesh, 16 to 20 mesh, or 14 to 16 mesh size millet seed particles versus whole millet. The sessions were conducted on four successive days (6 h/day). The food consumption from each food cup was measured daily so that daily preference scores could be calculated using the formula

$$\text{percent preference} = \frac{\text{ground millet consumed (in grams)} \times 100}{\text{total millet consumed (in grams)}}$$

A two-way repeated-measures analysis of variance [3 (particle size) by 4 (day)] was performed on the resulting data to test for differences among bird groups [7].

Bait Pellet Hardness Evaluation

A second group of 18 male quelea was used to assess the effect of bait-pellet hardness (that is, texture) on bait acceptance. Four levels of pellet hardness were produced by finely grinding whole millet into a flour, adding 10% weight/weight bentonite, and then adding different levels (weight/weight) deionized water: 16, 14, 10, or 8%. After being thoroughly mixed, separate batches of material were uniformly pelleted using a California Laboratory[2] Pellet Mill (Type CL-3). The resulting 8- to 16-mg pellets were air-dried in a laboratory oven at 50°C overnight. The hardest pellet formulation (16% moisture) was chosen as the standard material. Decreasing hardness of pellets was a direct function of the moisture level used in the mix before pelleting, so that the order of pellet hardness was 16 > 14 > 10 > 8%.

Essentially the same cage and preference test adaptation procedures were followed as were outlined in the section on bait particle size evaluation. All the birds were adapted to feeding from the automatic preference test devices and were preexposed to an equally proportioned mixture of the four pellet hardness levels for five days. The birds were then randomly divided into three groups ($n = 6$/group). Each group was given four successive days of 6-h preference testing in which the food choice was standard pellets (16% moisture) versus a softer pellet material (14, 10, or 8% moisture). Preference scores for each bird were calculated each day. These scores were subjected to a two-way repeated measures [3 (pellet hardness) by 4 (day)] analysis of variance [7].

Bait Color Evaluation

Four millet seed colors (red, blue-green, orange, and yellow-green) were used for the preference tests with 6-mg hulled proso millet (yellow) as the standard. Durkee's[2] food coloring (3 drops per 50 g millet) was used to achieve a uniform color coating on the seed. Sixteen male quelea were first exposed to an equally proportioned mixture of the five colors of millet seed over a period of five days and then randomly divided into four groups ($n = 4$/group). The birds in each group were then given four days of preference testing (6 h/day). Standard yellow millet versus millet treated with one of the previously described colors were the two choices offered.

The percent preference scores were calculated daily. This procedure was then replicated with the same birds in each group and the light intensity in the

cages was reduced to about 0.13 cd/m^2 (0.04 millilamberts) as measured 30 cm from the food cups. Previous work [*2*] had indicated that this light level allows the quelea to feed normally with essentially no food color perception. This replication served as a control test for the possibility of the birds basing their preferences on taste or other nonvisual stimuli.

Test scores for the two replications were examined using a three-way repeated measures analysis of variance [that is, 4 (colors) by 2 (light levels) by 4 (days)].

Results and Discussion

Particle Size

The preference test results are summarized in Table 1. All the birds in the three groups preferred whole hulled millet to ground millet, regardless of particle size, and the standard deviations for these preference test results were quite low (~4 to 5%). This result was unexpected because extensive food habits work on quelea in Nigeria [*4,5*] indicated that quelea naturally prefer small (0.1 to 0.3 mg) grass and weed seeds over the larger cereal grains cultivated in farming regions. This natural preference, according to our data, is probably not based on seed size *per se* but on other factors (such as flavor, texture, color, nutrients, or seed head form). However, one previous report [*8*] has also indicated that quelea prefer 8-mg millet seeds to small grass and weed seeds (0.5 to 5.0 mg) or large wild sorghum seeds (5.0 to 20 mg) in laboratory preference tests.

Analysis of variance essentially confirmed the lack of significant preference for any particle size over whole millet. Particle size ($F = 0.787$, $df = 2/12$, $P > 0.25$) and day factors ($F = 0.356$, $df = 3/36$, $P > 0.25$) produced no de-

TABLE 1—*Percent preference response [mean ± standard deviation (SD)] of quelea for whole hulled millet when different millet particle sizes were used as alternative foods.*

Millet Particle Size					
0.3 mg (20 to 28 mesh)		0.6 mg (16 to 20 mesh)		1.8 mg (14 to 16 mesh)	
Bird No.	$\bar{X} \pm$ SD	Bird No.	$\bar{X} \pm$ SD	Bird No.	$\bar{X} \pm$ SD
277	91.7 ± 4.6	236	95.3 ± 3.9	271	95.4 ± 4.4
262	94.9 ± 4.7	270	97.3 ± 3.3	287	95.5 ± 4.0
239	94.0 ± 4.8	234	96.8 ± 4.9	214	91.5 ± 8.3
205	95.5 ± 5.9	224	96.3 ± 4.6	222	95.9 ± 3.5
206	94.6 ± 3.8	255	96.3 ± 2.7	223	95.7 ± 5.5
Group means	94.1 ± 4.8		96.4 ± 3.9		94.8 ± 5.1

tectable effects, and the size by day interaction term ($F = 0.020$, $df = 6/36$, $P > 0.25$) was also nonsignificant.

Conversely, the data indicate that hulled proso millet is highly palatable to quelea, and field damage reports [*1,6*] tend to support this conclusion since it receives intensive depredation throughout the crop maturation cycle.

Pellet Hardness

Quelea were indifferent (50% preference) when the harder 14 and 10% moisture formulation levels were tested against the standard 16% moisture formulation pellets. As the pellets were made softer (8% level), however, preference decreased. These data are shown in Table 2 with the softest pellet test group showing a mean preference of 38 ± 19%.

The analysis of variance on data for individual birds over the four test days yielded a significant pellet hardness by day interaction ($F = 3.15$, $df = 6/45$, $P < 0.05$), day ($F = 5.17$, $df = 3/45$, $P < 0.01$), and pellet hardness ($F = 6.95$, $df = 2/15$, $P < 0.05$) effects. The interaction term was primarily due to the lowered preference across days for the 8% moisture (softest pellet) group, whereas the 14 and 10% moisture groups showed relatively stable preference across days. The day effect was mainly attributable to the 8% moisture level test group showing an ordered decrease in preference from Day 1 to Day 4. The significant pellet hardness effect ($P < 0.05$) was also attributed to the soft pellet group birds. These data predict that weathering processes (sunlight, rain, temperature changes) that tend to soften dry pelleted baits will reduce palatability.

Bait Color

The color preference test results for 16 birds tested under two light intensities are shown in Table 3. The birds in the blue-green group were quite vari-

TABLE 2—*Percent preference response [mean ± standard deviation (SD)] of quelea for pellets formulated at various moisture levels with 16% as the standard for comparison.*

Pellet Moisture Level					
14%		10%		8%	
Bird No.	$\bar{X}$ ± SD	Bird No.	$\bar{X}$ ± SD	Bird No.	$\bar{X}$ ± SD
250	51.0 ± 14.4	284	47.1 ± 8.8	290	45.7 ± 11.3
248	54.4 ± 14.9	258	49.5 ± 8.4	238	31.8 ± 19.9
223	55.0 ± 3.9	257	56.6 ± 5.8	207	37.6 ± 4.3
215	51.0 ± 10.4	252	60.4 ± 10.9	221	19.7 ± 11.9
259	50.4 ± 10.7	208	53.8 ± 7.3	278	55.1 ± 16.0
226	56.0 ± 16.4	230	44.4 ± 12.4	291	37.0 ± 12.9
Group means	53.0 ± 17.7		52.0 ± 13.4		38.0 ± 19.1

TABLE 3—*Percent preference response [mean ± standard deviation (SD)] of quelea for standard-colored (yellow) millet when compared with four other bait colors in a two-choice test (high versus low light levels).*

Millet Seed Color							
Red		Orange		Blue-Green		Yellow-Green	
Bird No.	$\bar{X}$ ± SD	Bird No.	$\bar{X}$ ± SD	Bird No.	$\bar{X}$ ± SD	Bird No.	$\bar{X}$ ± SD
			High Level				
1	89.3 ± 13.1	5	99.1 ± 1.8	9	92.4 ± 5.4	13	88.0 ± 15.8
2	95.0 ± 6.8	6	51.1 ± 10.4	10	56.2 ± 42.7	14	92.6 ± 5.5
3	91.7 ± 7.7	7	76.7 ± 20.2	11	31.9 ± 45.3	15	84.0 ± 19.0
4	91.6 ± 12.9	8	41.2 ± 25.6	12	7.6 ± 9.2	16	88.3 ± 12.3
Group means	91.9 ± 9.6		67.0 ± 27.9		47.0 ± 42.9		88.2 ± 12.9
			Low Level				
1	66.9 ± 8.9	5	42.7 ± 20.5	9	63.8 ± 6.4	13	62.4 ± 14.5
2	55.0 ± 15.2	6	45.6 ± 29.2	10	52.0 ± 16.1	14	72.7 ± 12.7
3	68.6 ± 18.0	7	73.2 ± 19.3	11	60.1 ± 27.0	15	56.7 ± 14.6
4	49.4 ± 10.2	8	12.1 ± 7.1	12	79.5 ± 28.9	16	49.5 ± 18.2
Group means	60.2 ± 14.7		39.8 ± 30.8		53.9 ± 27.8		57.2 ± 21.5

able in their preference behavior over the four test days (standard deviation = 42.9%), which tended to obscure color group differences when the repeated measures analysis of variance was applied to the scores. In general, the red and yellow-green groups preferred nontreated standard yellow millet at higher light intensity and reduced their preference (toward 50%) as the light intensity was reduced. The orange and blue-green groups were indifferent in their preference behavior at both high and low light intensities.

The three-way analysis of variance only yielded one significant difference, light level ($F = 16.77$, $df = 1/12$, $P < 0.01$), indicating mainly that the red and yellow-green groups preferred the standard yellow millet at higher light level and reduced their preference toward 50% when the light level was lowered. Since the repeated measures design is quite conservative [*9*] in detecting main effects for treatments (because color as a main effect included both low and high light level data), another analysis was performed. When the high light level data were subjected to a 4 (color) by 4 (day) repeated measures analysis, the significance level of the color main effect increased, but not to within acceptable levels ($F = 3.46$, $df = 3/12$, $P < 0.07$). The lack of significance was probably due to large day-to-day and between-bird preference variations in the blue-green group.

Even though these individual bird variations tended to confound preference test results, the color-preference effect should be evaluated further in quelea using larger sample sizes. Color preference data can, of course, be influenced by background colors and prior taste associations with particular colors [*3,10,11*]. Our results were in general agreement with a previous laboratory test report [*8*] that indicated quelea prefer light-colored grains but not to the exclusion of the darker seed varieties.

General Conclusions

This series of tests was undertaken to assess the quelea's preference for different forms of a millet seed bait material and to evaluate a laboratory bait preference test method suitable for this species. The results indicated that unaltered whole hulled proso millet is very well accepted by the birds under a variety of test conditions and would probably serve as a well-accepted bait carrier grain for quelea damage control. Millet seed has been used for evaluating the repellent effect of methiocarb in laboratory tests [*3*], and field reports indicate that millet is readily damaged at all stages of development.

One aspect of palatability that was not assessed in these laboratory tests was flavor, and there is a general lack of studies [*10*] aimed at evaluating taste preference in birds. The color preference data gave some indication that the birds preferred yellow over red or yellow-green seed under laboratory conditions, but more research is needed to fully evaluate this factor. For quelea, the automatic preference test devices provided orderly and statistically analyzable data when used with 6-h test exposure periods followed by 3 h of mixed-grain

access per day. All the birds appeared in good health after testing and maintained normal body weights.

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Rodents

A. Daniel Ashton,[1] *John H. McCumber,*[1]
and William B. Jackson[1]

Use of Outdoor Pens in Evaluating Field Efficacy of Rodenticides

REFERENCE: Ashton, A. D., McCumber, J. H., and Jackson, W. B., **"Use of Outdoor Pens in Evaluating Field Efficacy of Rodenticides,"** *Vertebrate Pest Control and Management Materials: Fourth Symposium, ASTM STP 817*, D. E. Kaukeinen, Ed., American Society for Testing and Materials, Philadelphia, 1983, pp. 129–137.

ABSTRACT: While laboratory efficacy testing of commensal rodenticides is essential in the development of a compound, this environment does not represent the "real world" of rodent control. Thus testing under actual-use conditions is necessary. However, such field tests may be seriously compromised by peripheral, nonexposed animals moving into an area immediately after rodenticide exposure.

When information necessary for rodenticide development, formulation, or re-registration does not warrant a full-scale field trial, a simulated field trial, using an outdoor pen, may be applicable. It offers a small but restricted population of rodents [such as house mice (*Mus musculus*) or white-footed mice (*Peromyscus leucopus*)] and is less expensive.

Pen trials are confined field trials, limited in scope, subject to normal weather conditions, yet with environmental controls more akin to those of laboratory testing. Outdoor mouse pens, with harborage and food, enable introduced animals to establish a reproductively active population, which the investigator can regulate; and sensitive index methods can be utilized to monitor population density and activity.

Penned areas (about 232 m^2) served as the test sites. Pen walls (aluminum sheet metal) extended 20 cm into a stone trench and 51 cm above the ground. A 1-m-wide grass strip on the interior perimeter was kept mowed to deter mice from moving near the perimeter wall. An area outside the pen also was mowed. Straw bales were placed within the pen, and grass and weeds were allowed to grow, the combination providing excellent harborage for mice.

Wild house mice released in the pen were allowed to acclimate to the surroundings for at least 30 days. Sherman live traps were placed along both sides of the pen perimeter for monitoring rodent movement into or out of the test area. Large Tomahawk live traps also were placed along the exterior pen wall to protect against ground predators.

Pens of this nature can be used for determining rodenticide efficacy, the comparative testing of rodenticides, or comparing population indexes with populations of known size. Such pens also constitute an investigative tool to study population responses to a rodenticide or bait formulation.

[1]Director, Rodent Research Laboratory, technician, and director, respectively, Center for Environmental Research and Services, Bowling Green State University, Bowling Green, Ohio 43403.

KEY WORDS: efficacy, pen trial, rodenticide, house mouse (*Mus musculus*), white-footed mouse (*Peromyscus leucopus*), brodifacoum, bromethalin, vertebrate pest control

Laboratory efficacy testing of commensal rodenticides is essential in the development of a compound. Present protocols enlist the use of small cages for group testing of mice; small water troughs [61 by 76 by 122 cm (2 by 2.5 by 4 ft)] also have proven effective in our laboratory for this purpose. Rowe and Bradfield [*1,2*] have suggested using families of wild house mice (*Mus musculus*) in indoor pens for efficacy testing.

Unfortunately, efficacy testing in the laboratory environment does not represent the "real world" of rodent control. Additional testing under actual-use conditions is necessary. However, field tests may be seriously compromised by peripheral, nonexposed animals moving into an area immediately after rodenticide exposure.

When information necessary for rodenticide development, formulation, or re-registration does not warrant a full-scale field trial, a simulated field trial, using an outdoor pen, may be applicable. It offers a small but restricted population of rodents [such as house mice or white-footed mice (*Peromyscus leucopus*)] and is less expensive to operate.

Materials and Methods

Site Overview

Outdoor pen trials were conducted during the fall of 1979 and summer of 1980 at the Rodent Research Facility, Bowling Green State University. The facility, located in rural northwestern Ohio approximately 4.8 km (3 miles) northeast of Bowling Green, Ohio, has several buildings and pens located on the premises.

Penned areas (about 232 m^2 or 2500 ft^2) served as the test sites. Pen walls (aluminum sheet metal) extended 20 cm into a crushed stone trench and 51 cm above the ground. Sheet metal was fastened to 5- by 5-cm posts, secured in the ground every metre (Fig. 1).

A metre-wide grass strip on the interior perimeter was kept mowed to deter mice from moving near the perimeter wall. A similar deterrent was used by Cole [*3*] for *Microtus* studies. An area outside the pen also was kept mowed. Sherman live traps were placed along both sides of the pen perimeter for monitoring rodent movement into or out of the test area. Large Tomahawk live traps also were placed along the exterior pen wall to protect against potential ground predators, such as raccoons, opossum, and cats. Live traps were baited with fish-flavored cat food.

Straw bales were placed within the pen; grass and weeds were allowed to grow, the combination providing excellent harborage for mice (Fig. 2). Addi-

FIG. 1—*Outdoor Pen B with mowed perimeter strip, 508-mm (20-in.) sheet metal walls, and interior harborage (grass, weeds, and straw bales).*

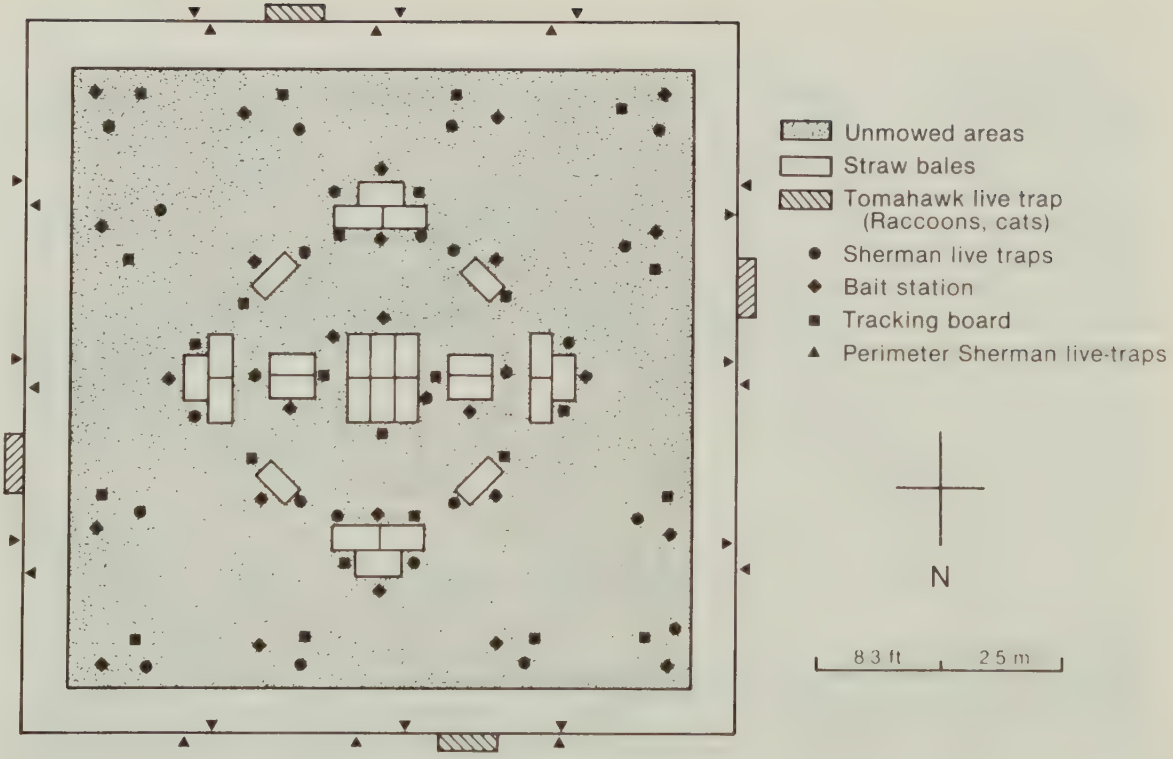

FIG. 2—*Harborage, bait station, and trap configuration used in Trial A.*

tional unbaled straw was piled in the center of Pen A (Trial A), thus concentrating the mouse population. Harborage, and hence the mouse population, was more evenly distributed in Pen B (Trial B). A density-frequency transformation of live-trapping data, as suggested by Caughley [*4*], confirmed these distribution trends.

Approximately 25 to 30 unmarked, adult wild house mice (sexes approximately equal), both laboratory bred (Pen A) and locally caught (Pen B), were released in the pens and allowed to acclimate to the surroundings for a minimum of 30 days. The mice set up residence in the straw and grass cover.

Field Trials

The field trials were divided into four phases: (1) pretreatment census, (2) treatment, (3) posttreatment census, and (4) snap-trapping. Three census techniques were employed during the pre- and posttreatment census: (1) trap-mark-release-recapture (T-M-R-R) methods, (2) bait consumption, and (3) tracking boards coated with carpenter's chalk [*5*]. Sherman live traps were set in the pen interior in grid fashion. Other census stations also were placed on grids but were shifted slightly to accommodate their proximity to straw bales. All points of the three censusing techniques were independent of one another. In one additional bait station, used as a moisture control, the bait was sealed off to prevent rodents from consuming it, but mice could still move through the station.

Pretreatment and posttreatment phases, each lasting 4 days, utilized cracked corn as the census bait; a 3-day snap-trapping phase commenced immediately following the posttreatment census. The treatment phase utilized Talon (0.005% brodifacoum) pellets in Trial A and lasted for 21 days. The treatment phase in Trial B lasted only 7 days and used EL-614 (0.005% bromethalin) toxic meal bait.

Results

Trial A

Information obtained from the T-M-R-R procedure [*6*] during pretreatment censusing indicated a mouse population of 42.5 (standard error = 11.6) animals. Days 1 and 2 were used as capture days; Days 3 and 4, as recapture days. A total of 45 different animals (22 males, 23 females) were trapped or recovered during the field trial from a population known to be reproductively active. Two pregnant females, both well advanced into the pregnancy, were trapped during the pretreatment census. One of these females was found dead on Day 6 of the treatment phase; the other female mouse was not recovered.

Several juveniles were recovered midway through the treatment phase,

suggesting there were nursing females within the population that were not trapped during the pretreatment census. The few mice captured and recaptured during posttreatment censusing make it impossible to estimate the population during this phase. However, comparison of total captures and trap success between pretreatment and posttreatment periods indicates a population reduction of 92.5% (Table 1). Census bait consumption was reduced in excess of 96% between pretreatment and posttreatment phases, while tracking board activity decreased 99% (Table 1).

Trial B

The mouse population in Trial B was evenly distributed in the habitat and slightly smaller than in Trial A; this was noted from the uniformity in daily takes at bait stations. Pretreatment census bait consumption also was lower in Trial B (Table 1).

Information obtained from T-M-R-R during pretreatment censuses indicated a mouse population of 27.1 (standard error = 2.3) animals. Actually, 27 mice (14 males, 13 females) were marked or recovered during the trial. No tracking board activity was measured during the posttreatment census. Overall, trapping and tracking board activity were reduced 100% from pretreatment levels; total census bait consumption declined 99%.

Discussion

Pen trials are confined field trials, limited in scope, and subject to normal weather conditions. Outdoor mouse pens, with harborage and food, enable introduced animals to establish a reproductively active population, which the investigator can regulate [7]. Sensitive census methods, such as a Lincoln index, can be utilized to monitor population size and activity.

Such an analysis indicated mouse populations of 42 and 27 for Trials A and B, respectively. Actually, 45 animals were marked or recovered during Trial A, and 27 animals were marked during Trial B; trapping activity also indicated the mouse population in Pen A to be half again the size of the mouse population in Pen B. Response discrimination due to sex was not apparent.

Tracking board activity is relatively even when considering total numbers of tracks ($A = 1092$, $B = 1068$). However, the number of tracks per board gives a better indication of relative activity ($A = 15.2$, $B = 10.7$) and maintains a consistent 3:2 ratio between trials.

Bait consumption ratios (Table 1) do not fit the 3:2 pattern and may be explained by the following observations: (1) The Pen A population was reproductively active and growing; several pregnant mice were observed during the pretreatment census. No pregnant mice were trapped in Pen B. (2) The average size of a mouse in Pen A was 24.9 g compared with 16.3 g for the

TABLE 1—*Summary data from two outdoor pen trials.*

Phase	No. Days	A (Brodifacoum)[a] Bait Consumption Total, g	A (Brodifacoum)[a] Tracks/ Board	A (Brodifacoum)[a] Trap Captures (Recaptures)	B (Bromethalin)[b] Bait Consumption Total, g	B (Bromethalin)[b] Tracks/ Board	B (Bromethalin)[b] Trap Captures
Pretest	4	1124	15.2	40 (11)	252	10.7	60 (33)
Test	21	569	...	...	54[c]	...	...
Posttest	4	44	0.2	3 (1)	3	0	0
Percent reduction		96	99	93	99	100	100
Snap-trapping	3	...	...	2 (0)	...	...	0
Carcasses recovered				19 (7)			

[a]Data collected from 18 sites.
[b]Data collected from 25 sites.
[c]Test terminated after seven days.

mice in Pen B, a result of the type of mice used in each trial. Pen A utilized laboratory inbred wild mice, animals generally more domesticated and less aggressive than wild-caught animals. The wild-caught animals in Pen B were quarantined in the laboratory, and then released in the pen; but territory establishment and concomitant mating behavior may have taken longer in Pen B. (3) Trial A was conducted during the fall, and much of the available weed seed may have been consumed, thus encouraging bait station activity. (4) Hoarding may have occurred, since Talon was used in pellet form.

The mouse population can be considered under control when average consumption per station decreases to approximately 0.5 g, an amount probably attributable to operator error. Therefore, the population was under control by treatment Day 2 in Trial B and Day 5 in Trial A. Unfortunately, the trials were not conducted during the same time and do not qualify as a comparative field trial [*8*].

No mice were found in the Sherman live traps adjacent to the perimeter fence. The walls and mowed grass served as sufficient deterrents to mouse movements. It can be concluded that neither immigration nor emigration occurred. Sensitive census techniques and the time necessary to obtain control are enhanced by the elimination of migration. The lack of migration also facilitates the comparison of the two field trials.

At times predators were significant problems. Local farm cats found the pen a source of food, but they were readily caught in the peripheral Tomahawk traps and removed. When laboratory-bred mice more than several generations removed from the wild were used, their responses were slow, and they fell prey to avian predators (especially crows). With protective traps in place and use of appropriate mice, no difficulties were encountered in the tests reported here.

Pen trials offer an intermediate step between laboratory group testing and full-scale trial. Rowe and Bradfield [*2*] noted that more than basic laboratory screening is beneficial, particularly when dealing with marginal rodenticides or when trying to determine optimum bait concentrations. Such trials also are useful in evaluating materials (such as zinc phosphide) that traditionally are difficult to test in cage environments. These limited trials are readily available and do not require extensive manpower. This also eliminates the need to scour the countryside for suitable mouse infestations and to have long driving distances between field sites and the laboratory.

Pens of this nature can be used for determining rodenticide efficacy, for the comparative testing of rodenticides, or for comparing population indexes with populations of known size. Such pens also constitute an investigative tool to study population responses to a rodenticide or bait formulation. This may be particularly helpful for analyzing rodenticides known to have difficulty in passing a "standard" laboratory efficacy test, yet effective in field ("real world") situations.

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A. Daniel Ashton,[1] *William B. Jackson,*[1] *and John H. McCumber*[1]

An Evaluation of Methods Used in Comparative Field Testing of Commensal Rodenticides

REFERENCE: Ashton, A. D., Jackson, W. B., and McCumber, J. H., **"An Evaluation of Methods Used in Comparative Field Testing of Commensal Rodenticides,"** *Vertebrate Pest Control and Management Materials: Fourth Symposium, ASTM STP 817*, D. E. Kaukeinen, Ed., American Society for Testing and Materials, Philadelphia, 1983, pp. 138–154.

ABSTRACT: Comparative field testing of commensal rodenticides is defined as a side-by-side testing of two or more materials under similar environmental conditions. This differs from comparison of field test data, where information is obtained at different times or under different environmental field conditions.

Comparative testing provides valuable information concerning advantages and limitations of rodenticides, including experience with special uses. This is useful for control operators when planning strategies for chemical and other control techniques in the development of integrated pest management. Comparative testing also may provide information pertinent to labeling instructions.

The design of a comparative field test should permit an unbiased evaluation of the rodenticides. The test site should be relatively homogeneous: a group of similar buildings, several contiguous city blocks, or a building which can be divided into comparable units. The use of isolated areas would be ideal but not essential if precautions are taken to avoid or limit rodent movements between areas. Equivalent pest infestations in all areas, although desirable, also are not essential if the censusing techniques are sensitive to the relative density. Use of two or more censusing techniques is desirable to prevent method limitations from biasing the test results. This permits greater flexibility in data analysis should one of the census methods prove inappropriate or unmanageable.

The test should include a control group or population, if possible. This control area (population) can be an untreated area or, in the case of a warfarin-resistant rat or mouse population, an area treated with a warfarin bait. Use of a control provides baseline information on population patterns within the test environment.

Included for discussion are comparative tests on Norway rats (*Rattus norvegicus*) in urban habitats and tests on rural resistant house mouse (*Mus musculus*) infestations. Rodenticides in pelletized and meal formulations were utilized as place pack and bulk

[1]Director, Rodent Research Laboratory, director, Center for Environmental Research and Services, and technician, respectively, Center for Environmental Research and Services, Bowling Green State University, Bowling Green, Ohio 43403.

baits. Compounds utilized during these evaluations include warfarin (0.025% bait), bromadiolone (Maki) (0.005% bait), brodifacoum (Talon) (0.005% bait), pyriminyl (DLP-787) (10% tracking powder), and bromethalin (EL-614) (0.005% bait).

KEY WORDS: rodenticides, commensal rodents, warfarin, bromadiolone, brodifacoum, pyriminyl, bromethalin, Norway rat, house mouse, field test, pest management, vertebrate pest control

Comparative field testing of commensal rodenticides is defined as a side-by-side evaluation of two or more materials under similar environmental conditions. This differs from comparison of field test data, where information is obtained at different times or under different environmental conditions.

Comparative field trials conducted in England [*1–3*] utilized an entire farm as a unit rather than parts of a farm or contiguous city blocks [*4*]. Drummond and Rennison [*5*] utilized a graphic method of monitoring treatment progress and a statistical approach to evaluation of rodenticide efficacy. Rowe and Redfern [*6*] discussed comparative toxicity of rodenticides to house mice in the laboratory, and Rowe and Bradfield [*7*] conducted comparative acute and chronic toxicity tests on confined colonies of house mice. However, only the latter laboratory method deals with side-by-side testing of rodenticides in a homogeneous environment. The present paper discusses comparative field testing of rodenticides in infestations of anticoagulant-resistant house mice (*Mus musculus*) and Norway rats (*Rattus norvegicus*).

Materials and Methods

Rural Site Overview

A chicken-rearing farm in rural northeastern Indiana served as one site for a comparative field test of commensal rodenticides. Trials were conducted on anticoagulant-resistant house mouse populations during the summer and fall of 1981.

The farm comprises one newer, single-story barn (Building A) and two older, two-story rearing barns (Buildings B and C). Approximately 9000 chickens are housed in Building A; 5000 and 6500 hens are housed in Buildings B and C, respectively. The buildings are divided into two sections, with the northern sections (about 5% of the floor space) serving as storage areas. Tools, equipment, and large amounts of cardboard are present in Building A. Food bins, present on the second floors of both two-story buildings, were each filled with three tons of chicken feed every three days. The one-story building had an external metal food bin. The deep-litter chicken-feeding operations, which last 20 weeks, are predominantly automatic.

While house mouse activity was observed throughout the buildings, most activity occurred in the feed and storage areas (north ends). Heavy house

mouse activity was also observed in the attic of each building, with the areas of heaviest activity in Buildings B and C being immediately above the grain bins.

The chicken farm has a history of prolonged exposure to warfarin baits. Approximately 115 kg (250 lb) of warfarin bait were eaten by mice during a six-week period in May and June 1981 with no apparent effect on the mouse populations. Sporadic and insufficient baiting during recent years has enabled a resistant population to become established. Effective control also is made difficult by highly competitive food sources, use of poor-quality baits, availability of extensive harborage, lack of natural enemies, and poor building maintenance.

Mice were trapped in each of the barns during April and June 1981 and returned to the Rodent Research Facility at Bowling Green State University. Standard World Health Organization (WHO) 1970 tests performed on these mice determined 40 out of 52 (77%) to be resistant to warfarin [*8*].

Trial I

Trial I was designed utilizing a rigidly structured protocol calling for pretreatment (four days), a three-day lag, treatment (22 days with censusing every Monday, Thursday, and Sunday), a three-day lag, posttreatment (four days), and snap-trapping (three days). Three census techniques [bait consumption, trap-mark-release-recapture (T-M-R-R), and tracking boards] were utilized during the trial.

The trial was divided into three tests. Building B was utilized to measure the effectiveness of bromethalin (an experimental-use rodenticide from Eli Lilly); Building C was utilized to evaluate brodifacoum (from ICI Americas). The third barn (Building A) served as a control house and was baited with the warfarin bait previously used on the farm.

Nineteen bait stations were used in each building during the treatment phase; one additional station per building was a moisture control protected from the mice. The bait was replenished to 100 g per station only on census days. Additional bait could not be added to high-activity stations due to a computerized data format restriction. (The significance of this restriction will be discussed later.) Place packs of bait material were used in the attics of each building, thus enabling the operators to provide toxic material to mice in 40 to 50% of each building. Censusing was limited to approximately 5% of each building, because the chicken-occupied areas were avoided.

Trial II

Trial II was initiated 44 days after completion of Trial I and utilized only Building A (treated with bromethalin) and Building C (treated with brodifacoum). Chickens were present only during the snap-trapping phase of

these tests, and the buildings earlier had been subjected to a moderate cleaning in preparation for new chicks. The census techniques were identical to those of Trial I, but the census points were redistributed to cover the entire first floor of Building A and all three levels of Building C. Pretreatment and posttreatment censuses lasted for four days, but lag phases were omitted. The treatment phase was to last until a marked reduction in bait consumption was obtained in both buildings. Treatment censusing was conducted weekly (less frequently than in Trial I), and toxic bait was provided in excess of need in the bait stations; the attic in Building A was baited with bromethalin in place packs. Also, the computerized data format was not utilized in this trial, providing greater flexibility in data collection.

Urban Site Overview

Comparative field tests on urban populations of anticoagulant-resistant Norway rats (*R. norvegicus*) were conducted in Chicago [*4*]. A 20-block area served as the test site for comparison of brodifacoum and bromadiolone bulk pellet formulations and bromadiolone meal in place packs. Four blocks were treated with each of these rodenticides; in another four-block area, burrows were treated with pyriminyl tracking powder. The fifth group of blocks served as an untreated control (Fig. 1). Only one census technique, active burrow counts [*9*], was utilized during this trial. Censusing and baiting programs were structured around city work schedules and rain interruptions. Baitings, carried out by city crews, were attempted approximately once a week but were not restricted to a specific day.

Results

Chicken Farm

Buildings B and C, structurally similar, were selected as comparative test sites; Building A was used as the warfarin-treated control area. The attics of Buildings A and C were filled with 15-cm (6-in.) fiberglass insulation, whereas corkboard was utilized for insulation in Building B. This did not appear to be a major variable at first, but later analysis of T-M-R-R data indicated that the larger mouse population in Building C (see Table 2) was due primarily to the increased mouse harborage afforded by the fiberglass insulation.

Bait consumption patterns are an index to the mouse populations in the census areas. Pretreatment consumptions indicated well-established populations in each building (Table 1). Treatment in Building A with warfarin had little visible impact on the population (Fig. 2). In fact, the daily consumption per station on Day 14 had increased 41% over the pretreatment average. Rowe and Bradfield [*10*] noted similar increases when using warfarin baits

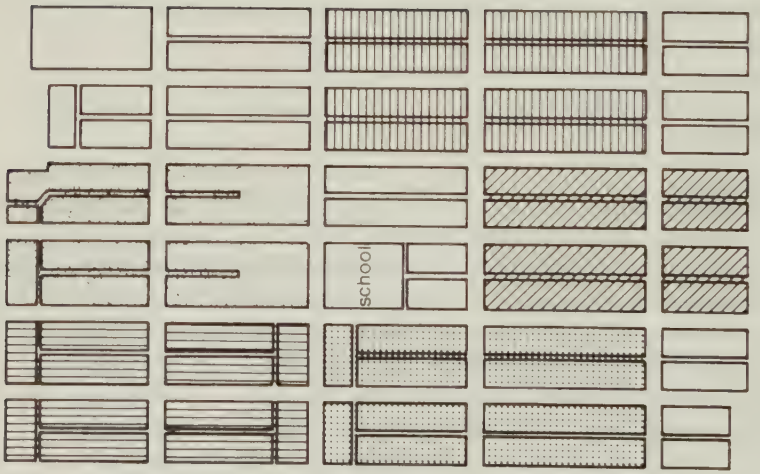

FIG. 1—*Twenty-block test area in Chicago (Lawndale).*

TABLE 1—*Summary of house mouse* (Mus musculus) *bait consumption activity during Trial I, Decatur, Ind., chicken farm.*

Phase	Date (1981)	Building A (Warfarin) Bait Consumption, g Total	Building A (Warfarin) Bait Consumption, g Weekly	Building A (Warfarin) Average Daily Consumption/ Station, g	Building B (Bromethalin) Bait Consumption, g Total	Building B (Bromethalin) Bait Consumption, g Weekly	Building B (Bromethalin) Average Daily Consumption/ Station, g	Building C (Brodifacoum) Bait Consumption, g Total	Building C (Brodifacoum) Bait Consumption, g Weekly	Building C (Brodifacoum) Average Daily Consumption/ Station, g
PRETREATMENT (15 STATIONS)										
Day 1	7/20	96.1	...	6.41	260.9	...	17.39	274.1	...	18.27
Day 2	7/21	148.5	...	9.90	371.8	...	24.79	232.3	...	15.49
Day 3	7/22	99.9	...	6.66	415.9	...	27.73	234.5	...	15.63
Day 4	7/23	152.3	...	10.15	446.6	...	29.77	278.9	...	18.59
Totals (average)		124.2	...	(8.28)	373.8	...	(24.92)	255.0	...	(17.00)
TREATMENT (19 STATIONS)										
Day 1	7/27	148.1	...	7.79	235.8	...	12.37	268.5	...	14.13
Day 4	7/30	595.1	...	10.44	174.0	...	3.05	695.4	...	12.20
Day 7	8/2	683.7	1426.9	11.99	79.8	489.6	1.40	272.6	1236.5	4.78
Day 8	8/3	201.5	...	10.61	7.2	...	0.38	40.9	...	2.15
Day 11	8/6	647.9	...	11.37	24.5	...	0.43	166.2	...	2.92
Day 14	8/9	664.1	1513.5	11.65	19.6	51.3	0.34	106.4	313.5	1.87
Day 15	8/10	179.5	...	9.45	21.9	...	1.15	40.3	...	2.12
Day 18	8/13	729.2	...	12.79	53.2	...	0.93	152.8	...	2.68
Day 21	8/16	750.2	1658.9	13.16	44.1	119.2	0.77	138.1	331.2	2.42
Day 22	8/17	302.3	...	15.91	12.8	...	0.67	86.8	...	4.57
Place pack consumption		1410.0	...		170.0	...		1060.0	...	
Totals		6311.6	...		842.9	...		3028.0	...	
POSTTREATMENT (15 STATIONS)										
Day 1	8/21	142.9	...	9.53	46.0	...	3.07	66.9	...	4.46
Day 2	8/22	257.2	...	17.15	75.3	...	5.02	122.9	...	8.19
Day 3	8/23	231.2	...	15.41	62.6	...	4.17	164.7	...	10.98
Day 4	8/24	235.4	...	15.69	56.4	...	3.76	147.5	...	9.83
Totals (average)		216.7	...	(14.45)	60.1	...	(4.01)	125.5	...	(8.37)
Percent reduction		(−74.4)	...		83.9	...		50.8	...	

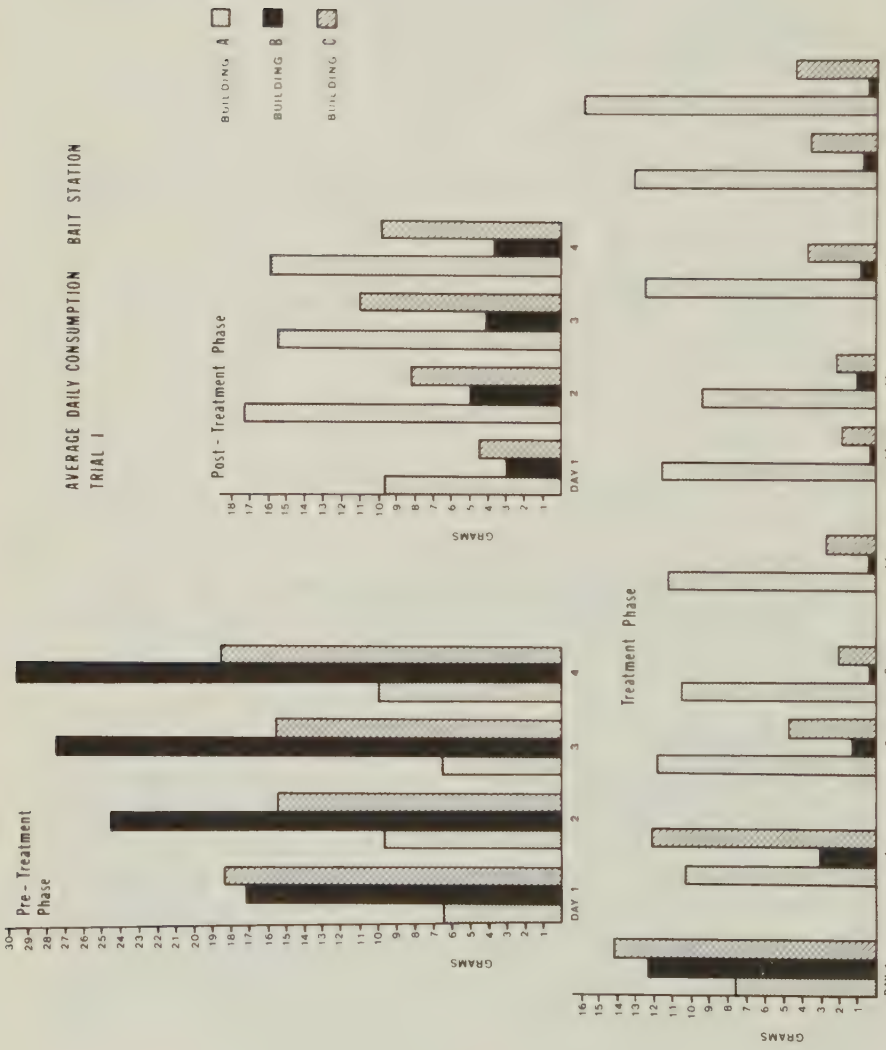

FIG. 2—*Average daily consumption at the bait station, Trial I.*

on confined colonies of warfarin-resistant house mice, which they attributed to the growth of young animals within the colony. The average daily consumption per station decreased 98 and 89% in Buildings B and C, respectively, during the same period (Table 1).

Rennison and Dubock [*11*] noted that the rats slow to feed on bait are the socially inferior animals, who are prevented from feeding until their social superiors succumb. Movement of the residual mouse population in Building C into the prime harborage and feeding areas (around the grain bins) was evident from continuous increases in food consumption after Day 14 of the treatment phase (Fig. 2). Trap-mark-release methods confirm this migration in two ways: (1) the animals marked during the pretest represented 30% of the carcasses recovered during the first week of treatment but only 2% of those recovered during the final two weeks of treatment; (2) no animals marked in the pretreatment census were captured during the posttreatment census. The apparent demise of socially dominant mice undoubtedly facilitated the mouse movements and the subsequent rise in food consumption during the posttreatment census (Fig. 2).

The partial control achieved in Trial I was due to the inability to get bait to the mice, since only a limited number of mouse-bait contact points was available; many potential sites could not be used because of disturbance and accidental ingestion of bait by the chickens. These problems were compounded by the limited baiting time and large mouse population.

Inordinately high movement levels rendered the tracking boards inappropriate as a census tool (Table 2) [*12*]. Many tracking boards were wiped clean of chalk overnight. The maximum activity category established was 30+ tracks per board [*13*]; normally this is adequate to quantify mouse activity, but in this trial it was surpassed. [In Trial II more than 150 countable tracks were visible on a 8- by 15-cm (3- by 6-in.) tile.]

Analysis of T-M-R-R data provides valuable information for assessing changes in population numbers. The Lincoln index is predicated on the random behavior of animals in relation to a trapping grid and is not directly suitable for determining population size in a structural house mouse infestation. However, utilization of such data [*14*] can provide indices to population size. Population indices during pretreatment census (Trial I) are 71, 155, and >500 for Buildings A, B, and C, respectively (Table 2). Because of the necessary restrictions on trap locations, such indices likely underestimate the actual populations. That the initial mouse populations were significantly reduced by the treatments in Buildings B and C is indicated by the lack of pretreatment-marked animals caught in the posttreatment census.

Mouse populations in Buildings A and C at the end of Trial I appear to be similar in size, with indices of 48 and 49, respectively. Since chickens were absent during Trial II, censusing was conducted throughout the buildings with optimum mouse-bait contact points. Initial population indices were 56 and 84 in Buildings A and C, respectively.

TABLE 2—*Summary of house mouse trapping and tracking board activity at the Decatur, Ind., chicken farm.*

Phase	Trial I						Trial II		
	Building A (Warfarin)		Building B (Bromethalin)		Building C (Brodifacoum)		Building A (Bromethalin)	Building C (Brodifacoum)	
	No. of Captures (15 stations)	Average Tracks/ Board/Day	No. of Captures (15 stations)	Average Tracks/ Board/Day	No. of Captures (15 stations)	Average Tracks/ Board/Day	No. of Captures (20 stations)	No. of Captures (36 stations)	Average Tracks Board Day
Pretreatment (4 days)	39	18	50	26	46	29	38	59	24
Population index	71	...	155	...	500+	...	56	84	...
Treatment	...	27	...	15	...	22	...	...	...
Posttreatment (4 days)	35	20	13	18	32	19	0	0	5
Population index	48	...	12	...	49	...	0	0	...
Snap-trapping (3 days)	50[a]	...	15[a]	...	54[a]	...	0	4	...
Percent reduction	32[b]	−11	92[b]	31	90+[b]	34	100[b]	100[b]	79

[a]Thirty stations.
[b]Calculated using the population index.

In Trial II, Building A was treated with bromethalin, Building C with brodifacoum. Bait consumption decreased 95% in both buildings. [The average daily consumption of 0.5 g per station (Table 3, Fig. 3) is attributed to operational error (<0.5%) and probably does not represent actual consumption.] That reduction was nearly complete is further supported by the 100% reduction in trapping success (Table 2).

Trapping data also indicated several demes of mice [*15*], which were not readily accessible during Trial I and were subordinate to the grain-bin population. However, the absence of chickens during Trial II enabled the operators to census bait the entire building, thus providing a more consistent data base.

The few mice observed during snap-trapping (Trial II, Building C) were caught near the first-floor door. Their presence is attributed to immigration from outside areas, since the farmer turned on the heaters and introduced chicks at this time.

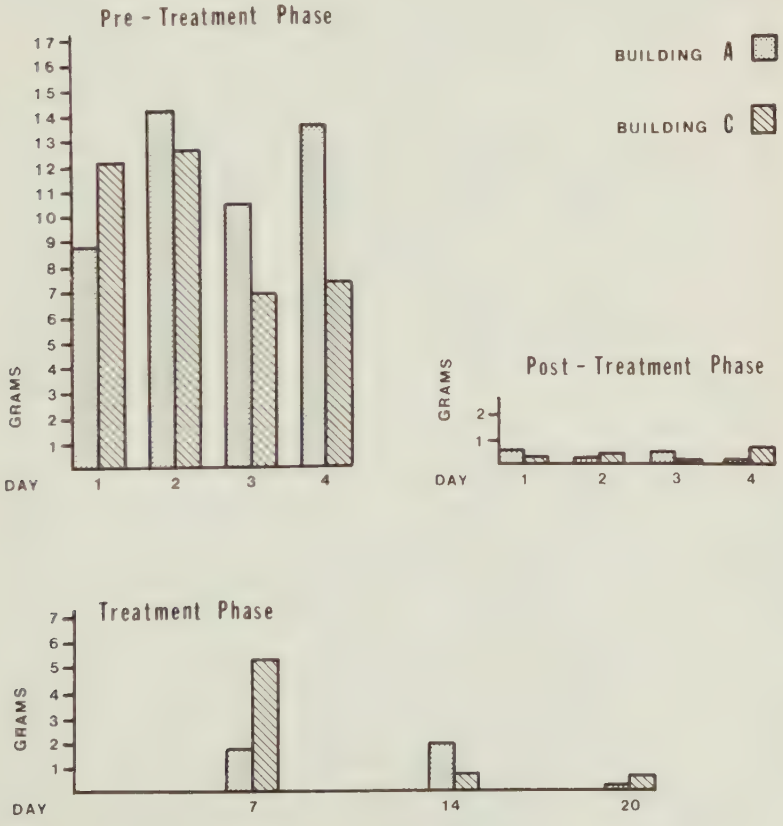

FIG. 3—*Average daily consumption at the bait station, Trial II.*

Urban Site

Sixty percent of the premises in the 20-block west-side Chicago area had active rat burrows. Treatment over three weeks with brodifacoum and bromadiolone baits produced active burrow reductions of 78 and 86%, respectively (Table 4). A single treatment with bromadiolone pellets produced a 60% reduction in active burrows. Burrows in one area were treated weekly with 10% pyriminyl tracking powder applied with an Acme foot-pump duster. After three weeks, only a 61% reduction in active burrows was achieved. [Note: The calculation of percentage reduction utilized comparison of "final survey" versus "first bait" data in an attempt to assess only active rat burrows. However, to be consistent with the calculations of the control group (initial versus final survey), the reduction would be 70% for brodifacoum, 50% for bromadiolone bulk, 89% for bromadiolone meal, and 60% for pyriminyl tracking powder.]

TABLE 3—*Summary of house mouse* (Mus musculus) *bait consumption activity during Trial II. Decatur, Ind., chicken farm.*

Phase	Date (1981)	Building A (Bromethalin) Bait Consumption Total (19 stations)	Building A (Bromethalin) Average Daily Consumption/ Station, g	Building C (Brodifacoum) Bait Consumption Total (36 stations)	Building C (Brodifacoum) Average Daily Consumption/ Station, g
		PRETREATMENT			
Day 1	10/13	166.6	8.77	434.5	12.07
Day 2	10/14	268.7	14.14	459.3	12.76
Day 3	10/15	202.2	10.64	297.7	6.88
Day 4	10/16	258.9	13.65	261.5	7.26
Totals (average)		896.4	(11.80)	1453.0	(9.74)
		TREATMENT			
Day 7	10/23	214.7	1.61	1278.4	5.07
Day 14	10/30	233.7	1.76	124.6	0.49
Day 20	11/5	10.6	0.09	97.4	0.45
Place pack consumption		165.0	...	...	...
Totals		624.0	...	1500.4	...
		POSTTREATMENT			
Day 1	11/6	15.8	0.83	14.3	0.40
Day 2	11/7	9.4	0.43	16.7	0.46
Day 3	11/8	16.1	0.85	9.7	0.27
Day 4	11/9	3.4	0.18	31.7	0.88
Totals (average)		44.7	(0.57)	72.4	(0.50)
Percent reduction		95.0		94.8	

TABLE 4—*Summary of block characteristics and the number of active rat burrows in four-block test areas of Chicago.*

Test Material	Number of Premises Surveyed	Percent of Premises Infested	Number of Active Rat Burrows						
			Initial Survey	First Bait	Second Bait	Third Bait	Second Survey	Final Survey	Percent Reduction
Talon pellet	137	65	337	448	400	261	...	100	77.7[a]
Maki bulk pellet	108	59	363	456	...	...	317	182	60.1[a]
Maki place pack	128	59	475	359	351	239	...	52	85.5[a]
DLP-787 tracking powder	158	52	472	482	368	257	...	189	60.8[a]
Untreated control	168	64	567	...	...	...	...	581	−2.5

[a]Calculated using the burrows active at the first baiting.

Discussion

Equivalent pest infestations in all sites, although desirable, are not essential if the censusing techniques are sensitive to the relative densities and population movements. While the design of a comparative field test should permit an unbiased evaluation of the rodenticides, it is not always possible to eliminate every bias.

Rennison [*3*] notes that the use of pretreatment and posttreatment baiting can bias results by conditioning the animals to enter bait stations. While this is true, bias in both such censuses should be consistent and, hence, not disturb the results. Also, a change in specific location of bait stations during the treatment phase might limit the conditioning bias.

The lag phase also can result in bias. When a population segment is eliminated by rodenticide baiting, an environmental vacuum is created; but since environmental conditions are unchanged, it is only a matter of time before the area is reinfested (for example, see Fig. 2, treatment and posttreatment, Building C). A lag phase allows time for immigration and thus may result in a false, nonefficacious declaration.

In contrast, continued treatment with toxicant until the food consumption decreases to a consistently low level will provide sufficient time for poisoned animals to die. Animals consuming a lethal dose (but with delayed death) will not be censused posttreatment. However, due to retraction of toxic baits, sublethally dosed animals, especially when using an anticoagulant, may recover sufficiently during a lag phase to be censused posttreatment.

High mortality during early treatment stages also favors immigration during a trial [*16*]. This is particularly true of tests with a limited treatment time and a large, high-density population. Establishment of buffer areas, physical barriers, or selection of isolated sites may limit immigration problems [*17*].

Bias also may be created by an unalterable, computerized data-collection format [*12*], prohibiting an increase in the daily bait allowance per bait station. Consequently, while several stations in Building C were being emptied overnight, the bait often could not be replenished for 72 h, thus limiting mouse/bait contact and, hence, control. Such a bias would not have occurred had the mouse demes been smaller or the initial bait placements larger than 100 g.

Another bias may result from environmental changes during field trial. A control (reference) population, be it in an untreated site or a resistant population treated with warfarin, provides information on responses to the environmental conditions during the field trial. This latter control test also provides a baseline for comparing new rodenticides against the warfarin standard. Thus, the use of a control population may reduce possible environmental bias while aiding data interpretation.

One of the better methods of censusing is direct observation, a valid method which often receives little support. However, unbiased sampling pro-

cedures must be developed and explained, with observational data recorded quantitatively. Thus, an experienced operator can provide valuable insight into population manipulation with rodenticides, and comparative field trials provide the operator with a direct understanding of population/rodenticide interactions.

What test design to use and whether to use one or more census methods in a comparative field trial must be decided by the operator. A Chicago rat infestation was related to backyard burrows [*4*]. In a series of tests, only one censusing technique, active burrows, was employed [*9,18,19*] due to time, environmental constraints, manpower limitations, and the large urban area. Other techniques, such as bait consumption, tracking boards, or live trapping, were inappropriate because of potential disturbance or loss of equipment.

Although use of two or more census techniques may make the results more "believable," the use of a single census method should not be immediately dismissed. Certain field test situations may lend themselves well to a one-census-method approach. The use of burrows in Chicago is such an example. It is recognized that burrow data only apply to the portion of the treated population using burrows. However, the use of such a method is not intended to measure actual numbers of rats; rather, burrow censusing is a measure of relative activity. That a non-burrow-bound method might show additional rat activity is irrelevant, since that population or portion of rats was not being censused initially. Undoubtedly, rats also may exist inside the structures, but the test was designed to measure the relative effectiveness of various rodenticides in the exterior baiting programs used in the Urban Rat Control Program. Furthermore, similar environments and the use of reference blocks limited the need for additional census techniques.

By comparison, the chicken farm mouse infestations were confined to relatively small areas. Initially, the trials were complicated by the presence of nontarget animals (chickens) and large mouse populations. The presence of chickens prohibited placement of census and bait points in many areas of the buildings, while the vertical movements of the mouse population made the infestations three-dimensional.

The complicated structure of the trial necessitated the use of more than one censusing method. Tracking boards proved unworkable (Table 2), due to intensive use and a statistical limit in evaluating the numbers of tracks per board. (An expanded evaluation scale would have been more satisfactory.) Two remaining techniques (trapping and bait consumption) provided a better understanding of the population/rodenticide interaction.

Analysis of Trial I bait consumption data (Table 1) suggests that the compound in Building B is more effective than the compound in Building C (84% versus 51% reduction). However, review of T-M-R-R data shows the mouse population in Building C to be at least three times that in Building B. The difference in population size, compounded by relatively few mouse-bait con-

tact points and waves of migration from other parts of the structure, resulted in a bias favoring the compound in Building B. The effects of underbaiting also increase with population size, because dominant animals protect bait from other individuals [*11,20*].

Mouse activity decreased sharply after the first day of treatment in Trial I, Building B (Fig. 2). The bait consumption activity remained low until residual mice moved into the census area two weeks later, when a second consumption peak is noted. Similar consumption patterns probably occurred in Trial II, Building A, but weekly censusing did not detect this (Fig. 3). Such patterns are to be expected with bromethalin, a single-feeding compound.

A slower reaction to rodenticide poisoning is expected with the new second-generation anticoagulants, as well as the conventional anticoagulants. In fact, bait consumption does not begin to fall until after Day 3 of treatment with brodifacoum [*7*], as illustrated in Fig. 2. No reduction was noted in Building A, which was baited with warfarin; this was expected, since the populations were known to be warfarin-resistant (Fig. 2).

Bromethalin in Trial I produced a more rapid knockdown of mice than brodifacoum. While brodifacoum also was effective in reducing the mouse population, the results were clouded by limited mouse-bait contact points that resulted in underbaiting and a tremendous migration of mice into the census area. Since warfarin was ineffective in controlling the resistant mice, treatment procedures provided an effective environmental control.

Lack of adequate control initially in Building C prompted concern for brodifacoum resistance and necessitated a second comparative field trial. Subsequently, control of warfarin-resistant house mice was obtained with both bromethalin and brodifacoum.

In the comparative field trials migration in conjunction with inadequate mouse-bait contact was responsible for lack of control with brodifacoum in Trial I. While control is possible by baiting in a limited area, such an approach necessitates a lengthier treatment phase. In contrast, the absence of chickens in Trial II permitted optimal mouse-bait contact and resulted in concomitant control.

Comparative field trials in an anticoagulant-resistant rat area of Chicago revealed efficacy differences among experimental formulations and delivery systems of value to manufacturers and formulators. A single application of bromadiolone pellets (in bulk) was marginally effective in reducing active rat burrows but comparable to pyriminyl tracking powder dusted into burrows. The latter material was less convenient and required more time for its application. [Note: pyriminyl (DLP-787 or Vacor) was removed from the market in 1979 by its manufacturer.]

Baiting of active burrows maximized rat-bait contact while limiting material waste. The use of active rat burrows as a single-census technique is efficient and suitable, because it focuses on a basic area of rat activity. A "back-up" technique is not needed.

Comparative testing provides valuable information concerning the advantages and limitations of rodenticides and techniques, including experience with special or optimal use patterns that can be incorporated into strategies for integrated pest management.

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Charles N. Stewart,[1] *Russell F. Reidinger, Jr.,*[2] *and J. Russell Mason*[3]

A Method for Inferring the Taste Qualities of Rodenticides to Rodents

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ABSTRACT: A method recently developed for the study of animal psychophysics was adapted for use in inferring the taste characteristics of rodenticides, as perceived by rodents. The method is based on generalization of learned food aversions to other materials having similar tastes. The potential of the method was illustrated in two experiments. In Experiment 1, five specimens of *Rattus norvegicus* (Sprague-Dawley) were conditioned to avoid the taste of strychnine (alkaloid) by pairing brief exposures to the strychnine with intraperitoneal injections of lithium chloride (LiCl). In subsequent 15-min drinking tests, the rats avoided mainly quinine sulfate and sucrose octaacetate [but not sodium chloride (NaCl) or hydrochloric acid (HCl), and sucrose only slightly] when compared with control rats. These results were confirmed in Experiment 2, and results from this experiment also indicated that sodium bicarbonate was not an effective agent for masking the taste of strychnine (that is, four rats conditioned with strychnine containing sodium bicarbonate still avoided quinine sulfate, compared with control rats in subsequent drinking tests, and four rats conditioned with only strychnine avoided quinine sulfate containing sodium bicarbonate in subsequent drinking tests). We suggest that the method has potential for use in assessing taste characteristics of rodenticides, as well as such bait additives as taste enhancers and masking agents, to rodents.

KEY WORDS: food aversion learning, rodenticide, sodium bicarbonate, strychnine, taste, taste masking, vertebrate pest control

Various approaches are used to gather information on the taste qualities (sweet, sour, salty, bitter) of materials to nonhuman species. All of the ap-

[1]Professor of psychology, Whitely Psychology Laboratories, Franklin and Marshall College, Lancaster, Pa. 17604.

[2]Wildlife biologist, U.S. Fish and Wildlife Service, assigned to the Monell Chemical Senses Center, Philadelphia, Pa. 19104, from the Denver Wildlife Research Center, Section of Supporting Sciences, Lakewood, Colo. 80225

[3]Assistant member, Monell Chemical Senses Center, Philadelphia, Pa. 19104.

proaches are necessarily indirect [*1*], and each has its own advantages and disadvantages. One approach, the simplest, is to base descriptions of taste qualities on the psychophysical responses of humans to the materials and to assume that human responses are similar to those of other animals. Unfortunately, the perceived taste of a material to humans may not reflect the perceived taste qualities of the same material to other animals. For example, cyclamate tastes sweet to humans at the same concentration that it is believed to taste bitter to rats and salty to hamsters [*2*].

In a second approach, an electrophysiological one, the animal is placed under anesthesia, and the chorda tympani or glossopharyngeal nerves, or single nerve fibers, are connected to microelectrodes in such a manner that summated single and multiunit electrical activity can be monitored. Differential neuronal activity in response to applications of tastants [such as sodium chloride (NaCl), sucrose, quinine hydrochloride, and hydrochloric acid (HCl)] to the tongue is then recorded. The method has been used to infer taste qualities of chemicals and to provide information on detection thresholds and identification thresholds of the compounds. Although used successfully in many studies of animal psychophysics [*1*], the method is limited by the complexity of experimental preparation, the time required to achieve large sample sizes, differential responses of the chorda tympani and the glossopharyngeal nerves to different taste qualities [*3*], and uncertainties regarding the relationships between attributes of neuronal activity (such as amplitude and frequency) and perception of taste qualities [*3*].

A third approach is based on work by Nachman and others. Nachman [*4*] and Nachman et al [*5*] presented lithium chloride (LiCl) in drinking water to rats who subsequently formed learned aversions specific to the taste of LiCl. Following this conditioning, the workers demonstrated that avoidance generalized to other compounds depending on the similarity of their tastes to that of LiCl. Thus, NaCl, which closely resembles LiCl in structure and probably in taste, was strongly avoided. Compounds such as potassium chloride (KCl) and ammonium bicarbonate (NH_4CO_3) were less strongly avoided. Nowlis and Frank [*6*] and Nowlis et al [*2*] have also used generalization of learned food aversions to describe taste qualities in rat and hamster taste perception. Animals were exposed to a taste [that is, a conditioned stimulus (CS)] and then sickened by injections of apomorphine hydrochloride [the unconditioned stimulus (US)]. In subsequent tests, Nowlis and co-workers recorded generalization of the learned aversions from the conditioned stimuli to a variety of compounds (test stimuli). One advantage of this approach is that it allowed the collection of taste information on large numbers of animals in a relatively short time. A limitation is that responses of the animals to the test stimuli may be confounded by differential hedonic responses (that is, unlearned likes and dislikes) to different taste qualities.

A fourth approach is to train the animal to indicate its discrimination between two taste qualities by some behavioral response, such as bar pressing or licking, in order to receive a food or water reward. The approach provides a

motivation for behavioral response other than taste quality and has been used by Morrison [7] to assess the taste qualities of chemicals to rats. The approach has the additional advantage of not requiring that the animal be made sick in order to display taste discrimination but the disadvantage of again requiring long periods of time to train and test large numbers of rats.

A better understanding of how rodenticides taste to rodents could lead to improvements in rodent control (see Ref 8). Two of the methods previously described have already been used to gather limited information on this issue. Richter [9] used preference tests to determine detection thresholds (that is, the minimum concentrations at which rodenticides could be detected) of seven rodenticides [alpha-naphthylthiourea (ANTU), arsenic trioxide, mercuric chloride, phenylthiourea, red squill, strychnine sulfate, and thiosemicarbazide] to *Rattus norvegicus*. He also requested a panel of 60 adult human volunteers to judge the bitterness of the compounds (the first approach). Kusano and colleagues have used the electrophysiological approach (the second approach described earlier), sometimes in conjunction with behavioral tests, to infer the taste qualities of thiourea derivatives (including ANTU) [10,11], thallous nitrate [12,13], and cycloheximide [14] to *R. norvegicus*.

We report here findings from an initial assessment of the use of generalization of learned aversions, the third approach, as a method to infer the taste qualities of rodenticides as perceived by rats. In Experiment 1, we used the method to describe the taste properties of strychnine to *R. norvegicus*, laboratory strain. In Experiment 2, we used the method to assess the effectiveness of sodium bicarbonate as a masking agent for the taste of strychnine. Strychnine was selected because Richter [9] had already provided information on the detection thresholds of rats to the compound. Sodium bicarbonate has been reported as a masking agent for the taste of the compound, but its function in this capacity has been questioned since Ward and Munch (using the first approach) [15] reported that sodium bicarbonate enhanced, rather than masked, the taste of strychnine in humans. We were particularly interested in the sensitivity of the method with regard to bitter tastes, in part because the method relies on consumption during drinking tests, and bitter-tasting materials are known to be relatively unpalatable to rats, and in part because many rodenticides, particularly the acute ones (including strychnine), are believed to have bitter taste components. Our choices of subjects and compounds were therefore logistically advantageous for an initial evaluation of the method but precluded immediate practical applications of the findings.

Materials and Methods

The subjects were adult male rats (*R. norvegicus*, Sprague-Dawley strain[4]), individually housed with free access to Wayne Lab Blox and to tap water, be-

[4]Perfection Breeders, Inc., Douglassville, Pa. References to trade names do not imply U.S. government endorsement of commercial products.

fore the experiments. The colony was maintained at about 22°C with a 12 h: 12 h light:dark cycle. For Experiment 1, we used 10 adult male rats weighing 450 to 550 g; for Experiment 2, we used 24 adult male rats weighing 396 to 473 g.

Chemical Stimuli

The following compounds, in distilled water, were used as conditioned stimuli: for both experiments, strychnine (levorotatory alkaloid—Aldrich, F. W. 334.42) at 0.0002*M*; for Experiment 2, a mixture of strychnine at 0.0002*M* containing sodium bicarbonate at 0.0016*M* and sodium bicarbonate at 0.0016*M*. In both experiments, LiCl (0.15*M*) injected intraperitoneally at 102 mg/kg body weight served as an unconditioned stimulus. In Experiment 2, NaCl (0.15*M*) injected intraperitoneally at 140 mg/kg was also used as the unconditioned stimulus for control rats. The following compounds, in distilled water, were used as test stimuli for both experiments: 0.1*M* sucrose (*S*), 0.1*M* NaCl (*N*), 0.01*M* HCl (*H*), and 0.0001*M* quinine sulfate (*Q*). In addition, 0.001*M* sucrose octaacetate (*O*) served as a test stimulus in Experiment 1, and 0.0001*M* quinine sulfate containing 0.0019*M* sodium bicarbonate served as an additional test stimulus (*BQ*) in Experiment 2. Sodium bicarbonate was added to the strychnine in the ratio of 2:1 (by weight) as reportedly used in bait formulations for control of ground squirrels.[5] The concentrations of the test stimuli were similar to those previously shown to be useful in generalization tests with other substances and rodents [*2,6*]. The strychnine concentration was selected so as to be equimolar to the highest detection threshold value reported by Richter [*9*], and thus one which would probably be tasted by all of the rats.

Adaptation

Before each experiment, the animals were adapted for at least seven days to a schedule of fluid deprivation, following the procedure of Nowlis et al [*2*]. The schedule was followed throughout the experiments. Each rat was given access to distilled water for 15 min (test period) during the first 6 h of the light cycle and to tap water for 30 min (hydration period) during the last 6 h of the light cycle. Fluids were presented in Richter tubes, calibrated in millilitres, and attached individually to the fronts of the cages.

Procedure

Experiment 1—For the last five days of adaptation, the rats were rank ordered according to their mean drinking scores during the test periods. Of those two animals with the lowest drinking scores, one was assigned randomly

[5]J. Bean, manager, Pocatello Supply Depot, Pocatello, Idaho 83201, personal communication, 1981.

to an experimental group and the other was assigned to a control group. Of those two animals with the next lowest drinking scores, one was assigned at random to the experimental group and the other to the control group, and so forth, so that the experimental group ($n = 5$) and the control group ($n = 5$) were balanced according to average fluid intakes.

On the day of LiCl dosing, the rats in the experimental group were allowed to drink the strychnine solution for 5 min during the test period, whereas the rats in the control group were presented with only the distilled water. Five minutes after drinking, each rat received an injection of LiCl. For two days following conditioning, the rats were given only tap water (*ad libitum*) to allow them to recover from the immediate effects of LiCl (or other aspects of the procedure) before testing the rats' responses to the solutions of tastants.

During the 15-min test period for five days thereafter, each rat was presented with 30 mL of a test stimulus (*H, N, O, Q,* or *S*), and consumption was recorded at the end of the period. One-bottle drinking tests were used instead of two-bottle tests, even though the former are less sensitive for detecting taste aversions [*16*], to encourage measurable consumption of *O* and *S*. Presentations of each of the five test stimuli were repeated for two additional weeks (following a Latin square design) so that all the rats had been exposed to each test stimulus three times over the course of three weeks.

Experiment 2—The same general procedure was used for Experiment 2. In this experiment, however, the last two days of adaptation (rather than the last five days) were used to rank order the rats, and the rats were assigned to six groups ($n = 4$ per group). Three of the groups (1 through 3) were experimental, and the others were control groups matched to experimental groups on the basis of drinking scores. On the day of conditioning, the rats were allowed to drink the following conditioned stimuli for 5 min during the test period: Group 1 and its control group, strychnine solution; Group 2 and its control group, strychnine solution containing sodium bicarbonate; and Group 3 and its control group, sodium bicarbonate solution. Five minutes after drinking, the rats in Groups 1 through 3 received injections of LiCl whereas the rats in the control groups received injections of NaCl (isotonic, 0.9% weight/volume). As in Experiment 1, the rats were then allowed two days to recover from the effects of LiCl. During the 15-min test period for five days thereafter, each rat was presented with 30 mL of a test stimulus (*BQ, H, N, Q,* or *S*) in one-bottle tests, and consumption was recorded at the end of the period. Taste presentations were repeated again for a second week (following a Latin square design) so that all the rats were exposed to each test stimulus twice.

Analysis

The data on consumption were converted to suppression ratios (defined as the amount of a test stimulus consumed by each experimental animal divided by the amount consumed by its control, the quotient subtracted from unity

and multiplied by 100), following the procedure of Nowlis et al [2] and Nowlis and Frank [6]. Although statistical analyses of suppression ratios are possible [2], mean suppression ratios of groups served adequately here as the bases for our descriptions of taste characteristics of the conditioned stimuli as perceived by *R. norvegicus*.

Results and Discussion

Experiment 1

Following association of the taste of strychnine with LiCl-induced sickness, the experimental rats suppressed their consumption of (that is, generalized their aversions to) *Q* and *O* compared to the consumption of these solutions by control rats (Fig. 1, Week 1). Avoidance of *Q* persisted during both additional exposures in Weeks 2 and 3 of the tests, but avoidance of *O* attenuated by the second exposure on Week 2. We concluded that aversions to strychnine generalized primarily to those substances that humans describe as bitter. The relatively rapid attenuation of avoidance to *O* could be explained as reflecting different strengths of taste of the two solutions or as representing two different types of tastes in the rat taste system, with *Q* more closely resembling the taste of strychnine than *O*.

Experiment 2

Following association of the taste of strychnine with LiCl-induced illness, Group 1 rats strongly avoided *Q* (Fig. 2). The percent suppression of *Q* intake was less in Experiment 2 than in Experiment 1, a finding which could be attributed to a change in the procedure in which the control group was given exposure to the strychnine unpaired with LiCl rather than to LiCl alone. The postingestional effects of the strychnine alone may have produced a weak aversion to that taste in the controls, thus lessening differences between the experimental animals and the controls. Because the rats in Group 2 avoided both *Q* and *BQ*, but not those in Group 3 who had been conditioned with only sodium bicarbonate as a CS, we suggest that sodium bicarbonate was ineffective in masking the taste of quinine. This notion is further supported in that Group 2 rats, conditioned with a mixture of strychnine and sodium bicarbonate, also suppressed their drinking of both *Q* and *BQ* to an extent similar to that demonstrated by the rats in Group 1 (Fig. 2). These findings are consistent with those of Ward and Munch [15] that sodium bicarbonate was ineffective in masking the bitter taste of strychnine to humans. Group 2 rats also avoided *N*, perhaps in response to the sodium in the sodium bicarbonate portion of their CS. Such a response was not observed in Group 3 rats, perhaps because the Na^+ taste was not potentiated by an association with strychnine (as it may have been for the Group 2 rats).

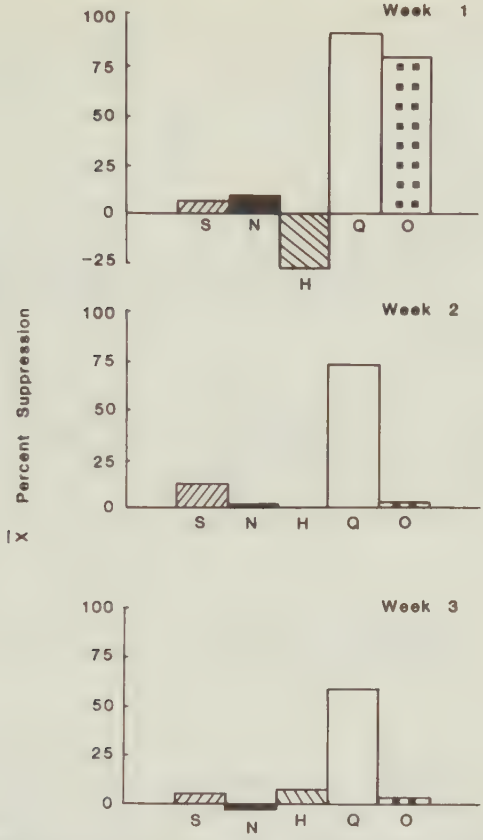

FIG. 1—*Suppression ratios, expressed as mean percentages (*n = *5), exhibited to the tastants sucrose* (S), *HCl* (H), *NaCl* (N), *quinine sulfate* (Q), *and sucrose octaacetate* (O) *during three 15-min drinking tests conducted over three weeks. Before the tests, the rats were conditioned to avoid the taste of strychnine.*

General Discussion

To our knowledge, the results represent the first assessment of the taste characteristics of a rodenticide using generalization of learned aversions. The finding that the taste of strychnine generalized primarily to *Q* and *O*, and that by inference the taste of strychnine appeared to be primarily bitter to the rats, is not surprising. These results are consistent with both Richter's findings [*9*], from his study involving a human taste panel, and the common categorization of strychnine (for example, Ref *17*) as having a "strong" taste, "normally objectionable to rats." The results are also consistent with the more general notion (for example, Ref *18*) that bitter tastes connote natural poison and are avoided by rats. We suspect, however, that studies with some other rodenti-

FIG. 2—*Suppression ratios, expressed as group mean percentages (n = 4 per group), exhibited to the tastants sucrose* (S), *quinine sulfate* (Q), *quinine sulfate containing sodium bicarbonate* (BQ), *HCl* (H), *and NaCl* (N) *during two 15-min drinking tests (the data for the two tests were combined) conducted over two weeks. Before the tests, the rats were conditioned to avoid the tastes of the following compounds: Group 1, strychnine; Group 2, strychnine containing sodium bicarbonate; and Group 3, sodium bicarbonate.*

cides and pest species will reveal more complex taste profiles, given the diverse chemical structures of rodenticides and the categorization of some as having only "slight" or "medium" tastes [*17*].

Although not surprising, the results are encouraging for several reasons. First, the method was sufficiently sensitive to detect differences between experimental and control rats in their drinking of presumably bitter-tasting test stimuli. Second, the method also appeared sufficiently sensitive to detect possibly subtle nuances in the taste of strychnine; that is, it tasted more like *Q* than *O* (at the concentrations used). Third, the method also provided support for the observation that sodium bicarbonate does not act as a masking agent for strychnine. Additional tests would be required, of course, to determine if pest rats respond in a manner similar to that of albino laboratory rats. However, some available evidence suggests that they would [*19*].

Obviously, the method requires additional refinement and evaluation before its full potential for use in the practical improvements of rodenticides and bait formulations is known. In interpreting our results from this study, we used the term "taste characteristics" loosely and cannot preclude the possibility that characteristics other than taste quality, such as texture, related odors, viscosity, or pH, served as conditioned stimuli for the learned aversions. Basic work is needed on the possible mediating role of such variables.

We have also explored the use of two different control group procedures, one (Experiment 1) in which the animals were given the illness-inducing agent (LiCl) alone (unconditioned stimulus), and the other (Experiment 2) in which animals were given a taste of the rodenticide (conditioned stimulus) alone. Both procedures were consistent and thus effective in comparison with the ex-

perimental groups, but we would recommend the use of the LiCl-alone control because it avoids the possibility of control animals forming an aversion to the taste of the rodenticide by virtue of its postingestional consequences.

Although we presented the rodenticide-CS in drinking water, we believe that the procedure could be modified to work with solid rodenticides and baits. For example, rodents could be presented with small amounts of a solid bait containing or not containing a rodenticide, then injected with LiCl and tested for generalization to liquid test stimuli, thus enabling one to determine the influence of the added rodenticide on the taste characteristics of the bait. By using analogous procedures, one might be able to test the effectiveness of various microencapsulating techniques or masking agents. By increasing the number of test stimuli, and by employing such procedures as multidimensional scaling in analyzing the suppression ratios [*6*], more refined descriptions of the taste qualities of rodenticides might be obtained. Such descriptions might be particularly useful in the development and assessment of complex bait formulations and in the improvement of prebaits through the addition of taste simulants, as suggested by Robbins [*20*].

In summary, the method provided results that were consistent with other studies on, and knowledge of, the taste properties of strychnine and sodium bicarbonate. The method appears to have potential for practical contributions to improvements in rodenticides and bait formulations.

Acknowledgments

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Karl D. Morris,[1] *Robert D. Proctor,*[1] *and Dale E. Kaukeinen*[1]

Design and Evaluation Criteria for Development of Toxic Wicks for Rodent Control

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ABSTRACT: Research suggests the feasibility of using wicks to deliver toxicants for rodent control, with subsequent rodent ingestion of the toxicant through grooming. Such wicks may overcome disadvantages of more conventional control materials, such as toxic baits and tracking powders for such applications as sites with little space or access for conventional baiting, with attractive alternative food sources, or where potential hazard and contamination are of particular concern.

Several innovations for developing and evaluating toxic wicks for rodent control are described. These methods are devised to determine optimum wick systems based on considerations of outer device construction, wick configuration and composition, and the nature and concentration of the toxicant in the device. Further, the delivery system to the rodent in relation to rodent susceptibility and behavior, wick placement, longevity, and safety are explored. Although the techniques illustrated involve a brodifacoum-containing wick device for commensal house mouse control, similar considerations and test procedures would have application in developing other toxicants and wick devices for rodent control.

KEY WORDS: rodent control, mouse control, contact rodenticide, toxic wicks, toxic devices, rodent control devices, mouse tube, Actimeter, rodenticides, house mice, vertebrate pest control, toxicants, contact toxicant protocols, motion detector, brodifacoum, simulated field methods, efficacy determination

Contact toxicants present an alternative to toxic baits in vertebrate pest control. Toxicants applied to the exterior of the animal may have particular potential for species or situations in which baits may be difficult to apply or are poorly accepted. Although contact toxicants can involve direct applica-

[1]Field biologist, formulation chemist (deceased), and technical representative, respectively, ICI Americas Inc., Goldsboro, N.C. 27530.

tion (such as sprays) to pest animals [*1*], the logistics of this approach with most pest vertebrates (except possibly large roosts of some bird species) make passive systems preferable. Passive contact toxicant systems require pest animals to encounter the toxicant themselves in the course of their normal movements or behavior. Paste toxicants, such as fenthion (Queletox or Entex), applied to beams, ledges, and structures have been studied for bird control [*2*]. Further investigations in bird control led to the development of toxic-wick perches [*3*] containing endrin or fenthion materials.

For mammals, most contact toxicants have involved the development of tracking powders, several of which (for example, anticoagulants, zinc phosphide) are currently marketed in the United States and elsewhere. Toxic foams (such as strychnine) have also been proposed, for control of mountain beavers, for example [*4*]. A 1080 grease has been used successfully for rabbit control in Australia [*5*]. Such contact toxicants have normally functioned by ingestion through subsequent grooming activity of the animal after exposure and body contamination. Such formulations are frequently placed in exposed areas and have had drawbacks in regard to potential nontarget hazard and area contamination. Furthermore, their ability to withstand climatic or environmental conditions has been limited. We describe here further development of an enclosed toxic wick rodenticide applicator device possessing several novel advantages for house mouse control.

Toxic wicks, as described herein for rodent control, function by depositing a toxic liquid onto the target animal. Intoxication occurs by ingestion during grooming. Such wicks are best used when protected by an outer enclosure.

Animal control devices of this and other types are frequently designed without full benefit of empirical knowledge gained through actual design evaluations. As such, they must, in many instances, be modified in actual use (such as expanded trap treadles [*6*]) to achieve the desired effectiveness. While control devices, especially nontoxic ones, are subject to fewer governmental regulations than toxic baits, individuals or groups marketing them are no less obligated to ensure a safe and efficacious product. User failure or fraudulent manufacturer claims (for example, with electromagnetic devices [*7*]) can create skepticism among users and a loss of credibility among professional animal management and control specialists, as well as the industries that supply them. Therefore, in order to properly develop and market these items, a comprehensive approach that encompasses product efficacy, construction, utilization by the target species, nontarget hazard, and cost is necessary.

Design and Evaluation Criteria

Overall Configuration

Recent developments in the United Kingdom [*8*] have involved careful determination of the optimum configurations and dimensions for such a wick station or covering tube, and for wick placement within such a device, to en-

sure utilization by and control of house mice. Research techniques previously described [9] enable rodent utilization of such a tube (see Fig. 1) to be more extensively evaluated, followed by an assessment of the overall construction and critical variations in the wick itself. The work described here used brodifacoum as a toxicant, as prior evaluation had suggested it to have optimum effectiveness among those toxicants tested [8].

Outer Construction

Control devices require some degree of durability. Safety to the user and nontarget animals is also a major consideration. The length of useful life and device safety are related to the choice of construction material. Cost constraints may preclude the extreme durability required to produce a device with a long use-life. Conversely, a pattern of servicing may favor lower initial cost by using replaceable devices or device inserts with a relatively short life span. Given that appropriate safety criteria can be met, both approaches can provide effective control in comparable situations through different schedules of application.

Other considerations affect the composition of structural components for the toxic wick delivery system. The impact resistance of the material should be considered in relation to the intended use. Additional factors are the suitability of the material for manufacture (for example, tooling, molding, and assembly); structural and chemical resistance to water, sunlight, and environmental pollutants; specific regulations or requirements for such devices; and, finally, the potential monetary return from sales of the device versus the development and production costs.

Toxic Wick Size and Configuration

The size and shape of the wick are important, as they determine product efficacy and longevity. The wick size and configuration are necessarily de-

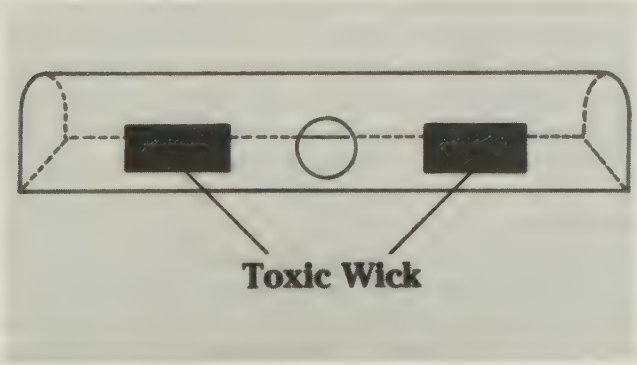

FIG. 1—*Toxic wick rodenticide applicator device.*

pendent, in part, on the outer shell configuration and wick composition. Several criteria must be met in order to obtain an optimum wick shape. Its size and shape must be such that target rodents are not restricted in passing over it. The shape must be designed so that there is a minimum differential effect for lower or higher weight animals, with a median effective shape that delivers the desired toxicant load at a rate based on pressure against the wick.

Toxic Wick Composition

The composition of the wick plays a major role in the length of life of the unit and is dependent upon the desired toxicant load as well as the desired life of the product. Significant factors in the choice of wick materials would be resistance to chewing and abrasion by the target rodents or breakdown of the material with age. Also important is the integrity expected of the wick, structurally and physically, under full toxicant loading. Incorporation of a nonvolatile oral contact repellent to reduce or prevent chewing of the wick by rodents might be considered or, alternatively, a screen covering for the wick to prevent chewing.

Transfer Toxicant Composition

Following determination of the composition and configuration of the tube, the actual transfer toxicant formulation must be selected. Obviously the choices made regarding this formulation are necessarily keyed to the active ingredient, the wick composition, and other factors. Of major concern is determining whether the toxic formulation, through olfactory or other cues, creates an aversion and reduces the animals' tendency to use the device. The length of active life of the wick is closely tied to characteristics of the transfer solution. Ideally, the transfer would occur at the same rate with the same animal, regardless of temperature, humidity, or wick age.

The product must remain stable for long periods of time *prior* to usage, since storage and transportation of the unit to the final user may occur several months after manufacture. The device must be able to withstand physical handling, whether it occurs during manufacture, shipping, placement, or in actual use. Flammability, toxicity, or irritability characteristics for the operator must be considered as well as any safety or regulatory concerns that may be applicable to the toxic formulation. Also, the ease of toxicant formulation and transfer onto the wick itself must be taken into account as related to large-scale production of the device.

Following the development of the transfer toxicant solution, it is necessary to "fine tune" the overall device for optimum performance. In such a system with a toxicant in a liquid formulation, product life and environmental and safety concerns relate to the volume of the toxicant placed within the unit. By increasing or decreasing the volume of toxicant utilized, the system could be adapted for a variety of use patterns.

Biological Tests

Initial prototypes require biological testing for efficacy. Laboratory testing at the authors' company involved six steps: determinations of tube utilization, initial applicator evaluations (free-choice pen trials), analytical technique development, control pass evaluations, advance prototype applicator device testing (small-pen trials), and advance prototype applicator device trials using large-pen evaluations. The device utilized in these trials measured 49.5 by 4.5 by 4 cm with a flat bottom. Two felt wick applicators were located approximately 10 cm from each end of the device and raised above the floor of the tube.

Development Trials

Applicator Device Utilization Trial

Initial evaluations involved mouse applicator devices which did not contain the transfer toxicant. This evaluation was conducted to verify that animals would make sufficient use of the device.

One group of five male and five female wild house mice, live-trapped from a local source, was utilized. Each animal was held for approximately a three-week period to prevent the use of pregnant animals as well as to acclimate them to captive conditions. The selected animals were weighed and toe-clipped prior to introduction into the test enclosure. This enclosure was a plastic laundry tub (U.S. Plastics Corp. Model No. 143675) which was 76.2 by 106.7 by 88.9 cm. The floor was covered with Bed-O-Cobs animal bedding (The Andersons, Maumee, Ohio), and two nesting boxes were provided (22.9 by 15.24 by 7.0 cm). A 4-L chicken waterer, placed centrally in the enclosure, served as a source of water for the animals. Ground Purina laboratory chow (*ad libitum*) served as the maintenance diet throughout the trial. All the animals were conditioned to the actual test environment for two days prior to the test. At Day 0 of the test phase, one blank nontoxic applicator device was placed into the test enclosure along one of the long walls and was mounted on a 76- by 5.1- by 10.2-cm piece of wood. The bedding in the enclosure was such that the top of the mounting board was even with the top of the bedding. A total of four Actimeter motion detection devices [*9*] were installed on the mounting board, with two being aimed across the entrance holes at either end of the device, and the remaining Actimeters inserted through the outer portion of the device and aimed at a point just in front of the applicator wick itself. Actimeters, as described by Kaukeinen [*9*], are infrared sensing devices which measure the combination of heat and body movement. These units have an internal memory and can register up to 9999 counts (activations). Counts are a relative index of activity and should not be confused with the total number of passages. Actimeters count activity at a certain point rather than necessarily revealing complete passes through the device. Each of the Actimeters was monitored daily. Food consumption was monitored daily

and spillage recovered and accounted for. The test period was maintained for four days.

Results—The activity recorded from the first day was considerably higher than that of subsequent days (see Fig. 2, left). By Day 3, the Actimeter counts leveled. This verified that the device was being utilized by mice and that further evaluations involving the addition of the transfer toxicant were warranted.

Initial Toxic Wick Evaluation

A second evaluation was conducted following the procedures mentioned previously, with the exception that the transfer toxicant was introduced onto the wick applicators themselves. The test phase was maintained for four days. A 0.25% solution of brodifacoum in polyethylene glycol 200 was applied to the wick at a rate of 7.9 g of solution per tube.

Results—As was seen in the first evaluation with the device with the blank wicks, rodent activity around and within the device with the toxicant decreased

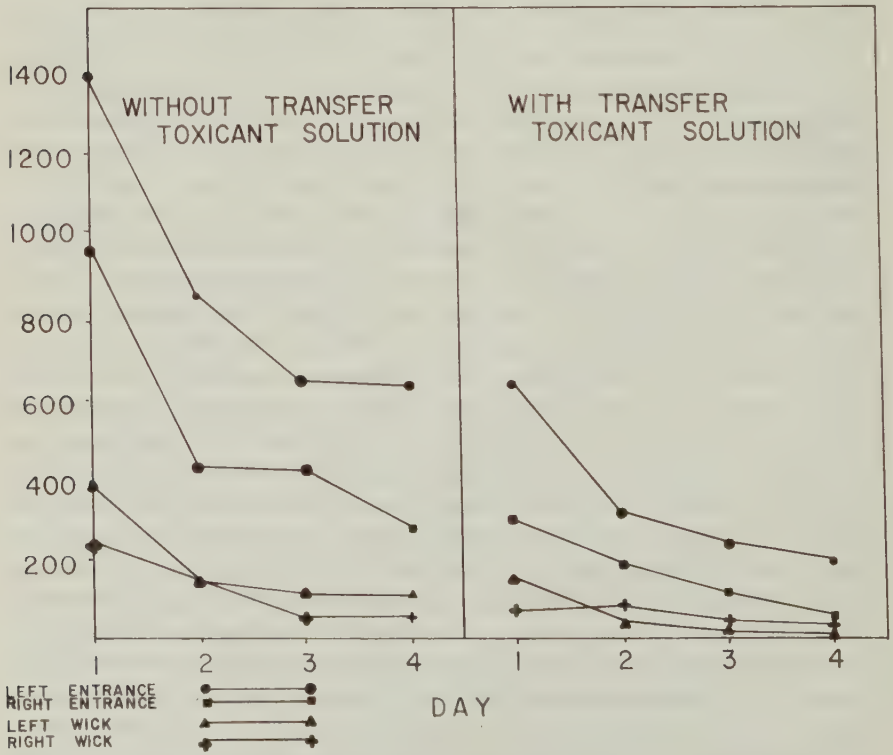

FIG. 2—*Activity count results (vertical axis) of initial testing of the applicator device utilized with and without transfer toxicant solution by wild house mice.*

as the mice became familiar with its presence in the enclosure (see Fig. 2, right). These results indicate that the incorporation of toxic wicks affected device utilization somewhat. However, as mice continued to utilize the toxic tube frequently, achieving mortality in a single lethal pass was unnecessary. Before additional efficacy research, an analytical technique to determine wick and toxicant characteristics more precisely was necessary.

Analytical Technique

Following these preliminary studies, it was necessary to determine the amount of active ingredient that was being administered to the animals per pass over the wick. We devised a method of determining mouse uptake by using a dye tracer technique as a simpler approach than the application of high-pressure liquid chromatography to mouse pelage or whole mouse carcasses [*10*]. The wick solution contained the identical components of the proposed toxicant solution, but with rhodamine B replacing the active ingredient. Each wild mouse was allowed to pass of its own accord over the wick containing 10% by weight rhodamine B solution for a total of 20 times. The animal was then placed into a bell jar and rinsed a second time with approximately the same amount of water. Both rinses were poured together and diluted in a volumetric flask to 250 ml. The dye concentration was measured by spectrophotometry. Some rinse samples were further diluted to obtain an absorbance value less than 1.0 to ensure linearity in correlating the dye concentration to absorbance.

The degree of saturation of the wick was varied to ascertain the optimum loading of toxicant solution and the rate of its depletion. This was felt to be critical, as it related directly to the performance and usable life of the tube. The levels of saturation were 10, 20, 40, 60, 80, 90, and 100%, with three wild mice being used at the 10, 20, 90, and 100% saturation levels. All the other levels had five mice. All the animals were exposed to fresh wicks. The toxicant carrier in these trials was oil based and stable over long periods of time, which precluded evaporation and other effects. Evaluations with toxic solutions subject to evaporation or breakdown would obviously need to incorporate methods to account for these possible sources of variation in product performance and in determining the animal uptake of toxicant from the device.

A series of dilutions of the rhodamine B solution was prepared to correlate absorbance at 555 nm to the volume of solution removed from the wick and subsequently diluted in the rinse water. The amount of material transferred to the animals per pass was calculated using the following formula:

$$R = \frac{\text{absorbance} \times \text{volume of rinse solution}}{\text{no. of passes} \times \text{calibration factor}}$$

where R = microlitres per pass.

After the dose rate, R, was determined, it was then possible to calculate the concentration of brodifacoum that would have been necessary by using the equation

$$C = \frac{K \times LD_{50} \times \bar{m} \times 10^3}{\bar{R} \times \bar{N}}$$

where

C = concentration of toxicant required, in micrograms per microlitre,
K = kill coefficient (number of LD_{50}s desired),
LD_{50} = median lethal dose, in milligrams of toxicant per kilogram of mouse,
$\bar{m}$ = average mouse weight, in kilograms,
$\bar{R}$ = average dose rate, in microlitres of toxic solution per pass, and
$\bar{N}$ = average number of passes over each wick.

As an example of this formula, take the following data:

K = 3 LD_{50}s,
LD_{50} = 0.3 mg toxicant per kilogram mouse weight,
$\bar{m}$ = 0.025 kg mouse weight,
$\bar{R}$ = 2 μL toxic solution per pass,
$\bar{N}$ = 5 passes, and

$$C = \frac{\frac{3\ LD_{50}}{1} \times \frac{0.3\ mg/kg}{1\ LD_{50}} \times \frac{0.25\ kg}{1} \times \frac{10^3\ \mu g}{1\ mg}}{2\ \mu L/pass \times 5\ passes/L}$$

$= 2.25\ \mu g/mL$
$= 0.225\%$ toxic solution.

Results—The amount of material removed from the wicks varied from animal to animal in proportion to the animal's size. It was determined that heavier animals apply more pressure to the wick itself, resulting in the removal of more toxicant (see Table 1). As larger animals require more toxicant for a lethal dose, this is a useful characteristic. With a 0.25% concentration of brodifacoum and an oral LD_{50} of approximately 0.4 μL/kg of brodifacoum, a desired rate of 2 μL per pass is felt to be necessary. For our purposes, pelage uptake was equated with toxicant ingestion, as preliminary data with the toxicant used suggested high grooming efficiency by mice. At 2 μL per pass, a 25-g mouse should obtain a lethal amount of material at approximately five passes through the device, traversing both wick applicators with each pass. Based on the results obtained from this work, it appears that this delivery rate can best be obtained with a wick initially saturated at 80 to 90% (100% wick saturation could give leakage problems). Although the amount of mate-

TABLE 1—*Sample results of analytical technique.*

Animal No.	Sex	Mouse Weight, g	Wick Saturation, %	Microlitres per Pass
1	M	14.1	10	0.00
2	F	31.8	10	0.21
3	F	26.0	10	0.04
4	M	20.6	20	0.04
5	M	19.9	20	0.04
6	F	12.6	20	0.03
7	M	20.9	40	0.08
8	F	19.3	40	0.08
9	F	22.4	40	0.15
10	F	15.7	40	0.07
11	M	23.1	40	0.09
12	F	16.9	60	0.08
13	M	15.2	60	0.07
14	M	17.3	60	0.31
15	F	10.9	60	0.01
16	F	21.1	60	0.48
17	F	18.8	80	0.28
18	M	21.7	80	1.96
19	M	14.8	80	1.49
20	F	16.6	80	0.81
21	F	19.3	80	1.75
22	F	23.8	90	11.62
23	M	16.8	90	6.31
24	M	18.1	90	3.06
25	F	19.0	100	8.97
26	F	15.7	100	5.69
27	M	15.2	100	5.22

rial removed at the various saturation levels appears to be relatively consistent, the dose decreased considerably as the level approached 60%. There seemed to be a fivefold decrease in the amount of material removed from 60% saturation (0.21 μL per pass) as opposed to 80% saturation (1.26 μL per pass). At 40% saturation (0.09 μL per pass), it would take 14 times as many passes (2 wicks per pass) to obtain one LD_{50} than at 80% saturation, suggesting that a device so depleted would be nearing the end of its useful life.

Controlled Pass Evaluations

In these studies, wild mice were allowed to run completely through the device and over both wicks a designated number of times over a period of several days. This allowed control of the number of exposures to toxic wicks and the actual length of exposure.

Wild house mice were collected from a swine production facility in Wayne County, North Carolina. These animals were held in the laboratory, sexes separate, for a three-week period prior to the test. As each animal was se-

lected, it was weighed and placed in a single-animal holding cage (Hoeltge-HB26 rat cage). The animals were then grouped into levels of three female and three male with an additional male and female serving as controls for each level. Each group was assigned either 2 passes, 5 passes, 10 passes, or 20 passes per day for a total of five days.

All the levels were acclimated to the laboratory conditions for four days prior to testing. Water was available *ad libitum* via water bottles fitted with sipper tubes and mounted onto the front of each cage. Ground Wayne Lab Blox served as the maintenance diet for the animals during the entire study. Food consumption was monitored daily, with adjustments being made for spilled food.

Following the conditioning period, each animal was allowed to pass through devices saturated with toxicant at 80% for the appropriate number of times designated for the group to which it was assigned. The mice were not allowed to groom until after they had completed the assigned number of passes, after which each was placed back into its holding cage. All the animals repeated the routine daily for five days. At the end of the treatment period, all the animals were observed for ten days or until death, when, in either case, each mouse was again weighed.

Results—All the animals died with the exception of one female in the two-pass per day level (see Fig. 3). The animals exposed to the higher number of passes per day died more quickly than those with fewer number of passes per day. The results are as follows:

No. of Passes	Mortality	Average Day of Death	Range
2/day	5/6	6.4	4 to 10
5/day	6/6	5.6	4 to 10
10/day	6/6	5.5	3 to 8
20/day	6/6	4.8	3 to 7

From this information, it would seem that repeated passages are indicated over a period of several days, given these wicks and wick loadings, in order for the target rodents to obtain a lethal dose of the transfer toxicant solution. This is preferable to a single lethal dose concept in terms of potential hazard in that less active ingredient is needed in the wick and less is deposited on target rodents. Greater wick concentrations might result in unnecessarily high doses received.

Advanced Prototype Device—Small Pen Test

After the efficacy of the wick system had been verified in the controlled pass evaluations, it was necessary to determine the effectiveness of the design

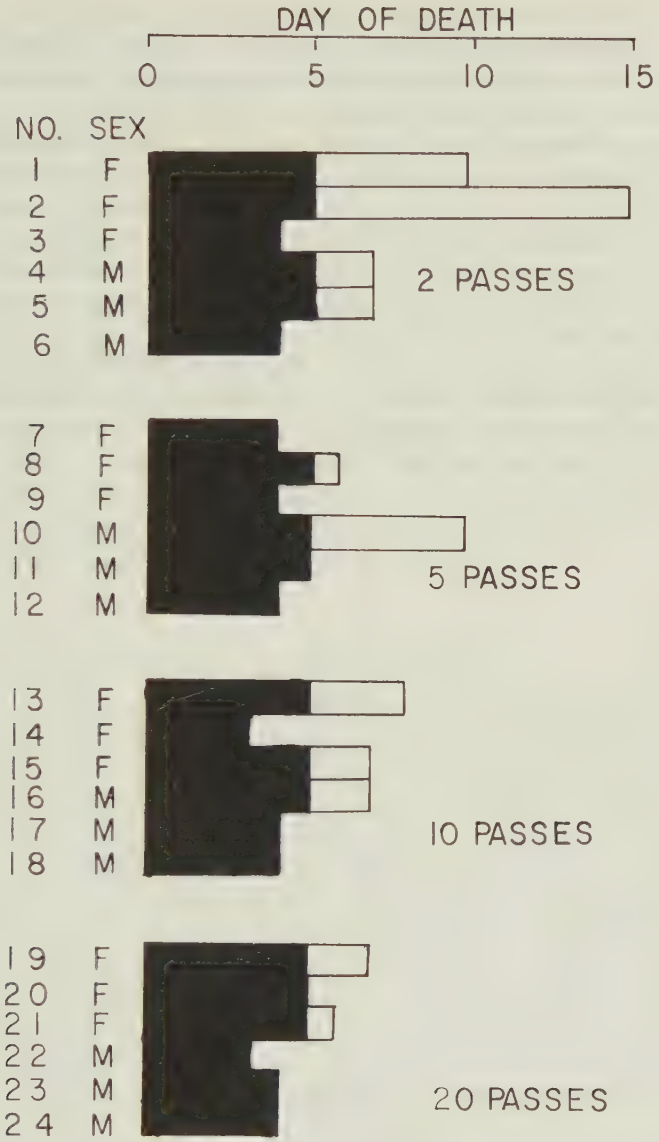

FIG. 3—*Results of controlled pass evaluations using the applicator device and wild house mice. The shaded portions of the graph indicate the period of toxicant exposure.*

in small enclosures with groups of wild mice. This allowed a determining of the group interaction toward the device, as the mice were given free access to the device during the treatment phase. This allowed further verification of the efficacy and design characteristics of the device.

Wild house mice were collected and acclimated as previously described. At

the start of the test, each animal was weighed and toe-clipped for future identification. Five male and five female mice were then placed into the test enclosure. Each bin was supplied with two large nesting boxes and Bed-O-Cobs animal bedding. Water was made available via a 4-L chicken waterer.

After introduction into the bin, the mice were allowed to acclimate for several days prior to the start of the test. Following the conditioning period, one device was placed along one wall of the bin. During all phases of this trial, food consumption was monitored daily. The mice were maintained on ground Wayne Lab Blox. The test period was maintained until all the animals died. Daily checks were made in the nesting boxes to recover any dead animals.

Results—As in the previous evaluations (Fig. 2, left) involving applicator devices that did not contain toxicant, activity around the entrances and over the toxic wicks was greatest on the day of introduction (Fig. 4). However, the total activity decreased daily for the remainder of the trial. Actimeter counts

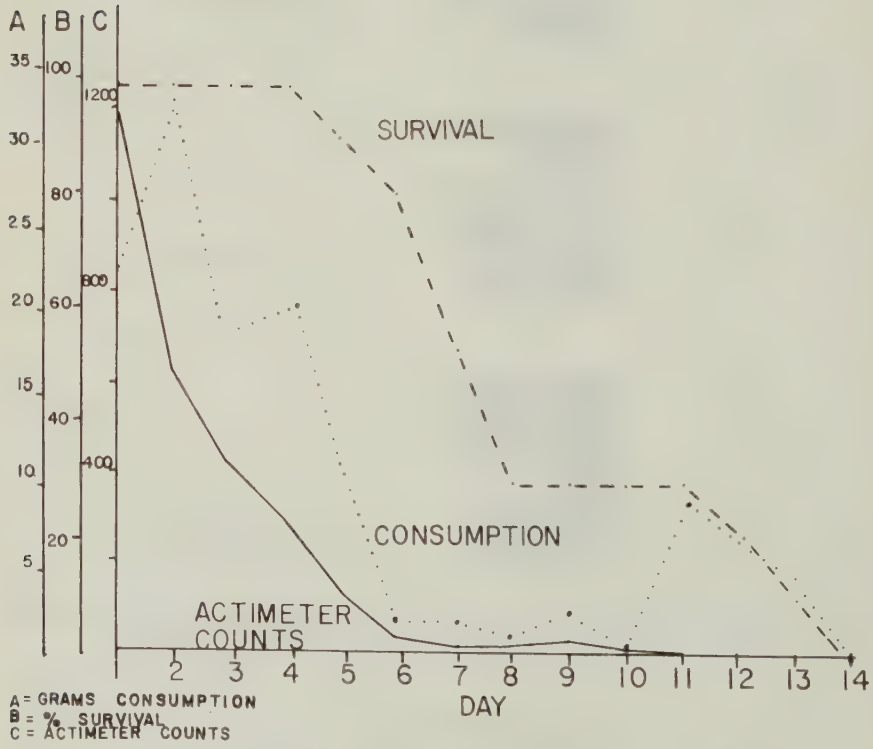

FIG. 4—*Consumption, activity, and survival of ten wild house mice in a small enclosure containing a single applicator device and alternate harborage.*

on Day 1 totaled 1180 counts and fell to 287 total counts by Day 4. Similarly, Maruniak et al [*11*] saw a decrease in urinary marking in male house mice following an initial exposure to an unfamiliar or novel object. Wolfe [*12*] also saw a decline in activity of wild house mice after exposure to a novel object and suggested that this is characteristic of their exploratory tendencies. Thus, the decrease in activity seems related to habituation rather than avoidance.

The habituation behavior may prevent mice from obtaining significantly higher amounts of toxicant than is necessary to produce mortality. In this particular investigation with ten mice, 100% mortality was reached with a prototype device, with a mean day of death at Day 8.7. The day of death for individuals ranged from 5 to 14 days.

From this study, it appears that utilization of the device occurs at such levels that control over a two-week period of a small stable population of mice can be expected. The mean day of death was similar to that seen in laboratory feeding studies involving 50 ppm brodifacoum bait [*13*]. This suggests that there should be no significant difference between the time to death for the two types of control applications of the same toxicant.

Advance Prototype Device—Large Pen Evaluation

The use of large pens was a necessary step in the development of the toxic delivery system. In order to verify efficacy as well as to develop the optimum usage pattern for the field, these studies allow a more natural environment for the mice, yet allow the researcher to control variables which are not easily manipulated in field situations. These factors include lighting, environmental noise, immigration, emigration, and human disturbance.

The enclosures used for this study were constructed of 16-gage sheet metal and were 3 by 3 by 1.2 m high in size. The interiors of the pens simulated a warehouse situation. On two standard shipping pallets were stacked four 23-kg bags of laboratory animal diet. Water was provided *ad libitum* via a 22-L chicken waterer centrally placed in the enclosure. Along the outside of one wall was a raised platform from which an observer could make visual observations without disturbing the animals. Mirrors assisted making observations.

In order to decrease disturbances, all windows within the building were darkened, and black curtains were placed over them. Additionally, a curtain was placed between the enclosure and the observation platform. A photoperiod of 12 h light and 12 h dark was maintained throughout the period of the evaluation. In order to make observations of the mice within the pen under "night conditions" when mice are most active, red fluorescent tube lights (Sylvania 75-W) were installed at ceiling level and were connected to a timer switch so as to be on during the 12-h "night" period. An AM/FM radio

was wired into the same time circuit as the white light. The combination of white light and sound from the radio was intended to discourage mouse activity during the "day" and to restrict their movements to within the pallets. An ultrasonic sound generator might also have been useful for this purpose if activated during "day" conditions, although this was not attempted in these experiments.

Two methods of population censusing were utilized in this evaluation. The first method, visual observation, was conducted by sitting behind a small opening in the blackout curtains on the observation platform and noting mouse movement under red light within the enclosure for a unit period (15 min). Various behaviors were noted, such as moving within a pallet, moving between pallets, and moving along a wall, and these were totaled for a daily visual activity count. The second method involved the use of Actimeters. The Actimeters used in this evaluation were fitted with power supply adaptors (Radio Shack Model No. 270-1552A) that converted the unit's power supply from 9-V transistor batteries to A-C current to prevent the loss of data due to premature battery failure. These adaptors were then plugged into an electrical filter (Radio Shack Model TRS-80 Line Feed) to prevent any power surges which might have interfered with proper Actimeter operation.

An Actimeter was placed on each corner of the two pallets, oriented so that it aimed at a point approximately 5 cm away from the end of the stacked commodities on the pallet. Each unit was fitted with a readout extension cable so that observations could be made from the outside of the enclosure to further keep human disturbances to a minimum.

In order to prevent mice from chewing into the power supply cord and the readout extensions, each Actimeter was fitted with a 61-cm piece of rigid plastic pipe (3.8 cm in diameter). All the cords were run down through the vertical pipe, which was fastened to the Actimeter with a U-shaped bracket. To prevent the mice from climbing the pipe and escaping, a 30- by 30-cm barrier of 0.3-cm Plexiglas was fastened to the top of the pipe.

At 24-h intervals, each Actimeter was read, and the total number of counts registered was noted. This followed the visual observation period to prevent causing disturbance of the mice which might influence the visual counts.

At the initiation of the study, ten wild house mice (five male and five female) were selected. All the mice were recent captures from a swine production facility in Wayne County, North Carolina. Each animal was weighed, toe-clipped, and placed into the test enclosure. Once a day, after introduction of the mice into the test enclosure, observation sessions were conducted. Visual observations were conducted for 15 min per day and were followed by Actimeter monitoring. All observations were conducted at approximately the same time each day throughout the trial. The mice were observed for six days prior to treatment. There were no observable deaths noted due to fighting.

Following the pretreatment observation period, two devices containing

toxic wicks were then placed into the pen along the inside edge of the pallets against the sacked material. To verify tube utilization, four additional Actimeters were placed into the enclosure and were aimed directly at each of the two wicks within the tube through two 1.3-cm holes drilled into the top of the device over the interior wicks. These units were powered by 9-V batteries. Daily readings were made from these units and recorded separately from other Actimeter counts that were collected.

The toxicant applicator devices were maintained within the test enclosure for ten days. At the end of the treatment phase, all the material on the pallets was thoroughly searched to recover dead mice or survivors. The mice recovered were identified and weighed.

Results—By comparing the last three days of the conditioning period with the last three of the treatment period of the large pen evaluation, reduction of activity can be determined (Fig. 5). Pretreatment visual observations showed a three-day total of 279 counts, while the three-day period at the end of the treatment showed only 11 visual counts, a 96% reduction in visible mice under red light conditions. Similarly, when the total Actimeter counts from

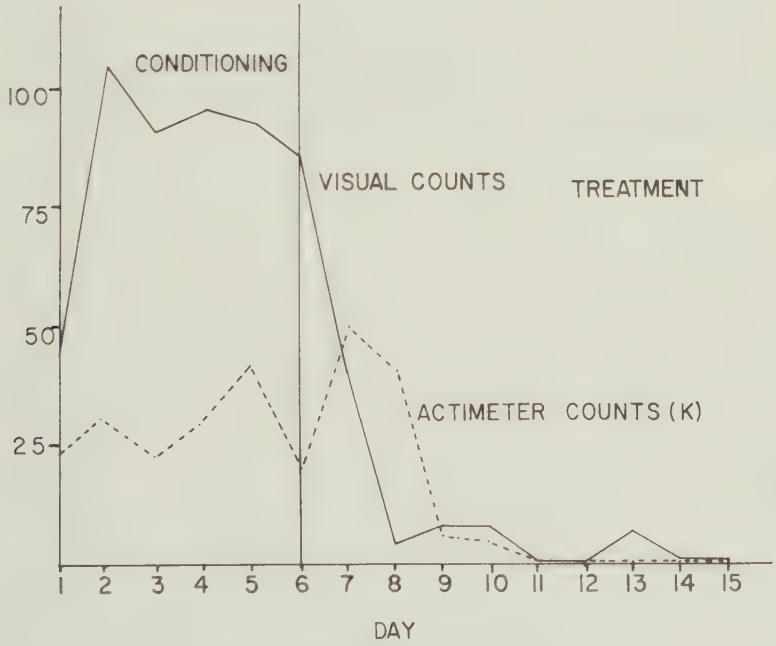

FIG. 5—*Activity as determined from visual and mechanical counts of a group of ten wild house mice in a large enclosure containing two toxicant applicator devices and simulated warehouse conditions. Actimeter count units are expressed in thousands.*

the eight units during the last three days of pretreatment were compared with the total activations registered during the last three days of the treatment, a 99% reduction in mouse activity was obtained. Ninety percent of all introduced mice died during the ten-day trial. Several mice were recovered from within the stored commodities, while the remainder were found in unexposed areas underneath the pallet, with the exception of one animal which was found near the waterer in an exposed location. This supports the contention that, in such infestations, some mice may be restricted to the pallet and are not as susceptible to the more conventional forms of baiting [*14*].

The total daily Actimeter count from the eight units during pretreatment was above 20 000 and increased to about 50 000 on the first day of treatment. This rate of increase was similar to that noted in the small pen evaluations. There was then an immediate decrease in Actimeter counts, which continued to Day 11 (Day 5 of treatment), when the total counts stabilized at less than 100 counts for the remainder of the study. Visual counts were stabilized by about Day 3 of the pretreatment period. After placement of the tube into the enclosure, visually determined activity steadily decreased, with no mice being observed on Days 11 and 12. On Day 13, nine visual observations were noted, which dropped to one visual count on Days 14 and 15. At this point, the study was concluded.

In this simulated experiment, the prototype device effectively controlled a small free-ranging population of wild house mice. The rapid population decline, as well as the ease of application, make the device favorable for this pattern of usage.

Discussion

These design and evaluation criteria should be useful for development of toxic wick devices. The toxic wick concept has considerable merit in that currently registered rodenticides may be incorporated into the wick system, thereby lessening the amount of time and expenditure necessary for registration. These devices have great potential for mouse control under certain conditions, including within stacked or palletized commodities, where conventional control techniques and measures are inappropriate, or in areas with large amounts of competitive foodstuffs, where baits may not be readily accepted. Ceiling areas and rafters would also be suitable areas for placement of such devices. The test and analytical methods described here would be valid for use in the development of other toxicants for incorporation into such devices and are offered to advance such rodent control applications, much as Nichols and Crabb have done for avian contact control devices [*15*] and Davis [*16*], Marsh [*17*], and Meehan [*18*] have done for rodenticidal tracking powders.

Subsequent evaluations, to be reported later, will involve field efficacy determinations in the United States with the modified rodenticide applicator device developed by the authors' company, using methods previously described [*9*]. A cardboard version of this device with a somewhat different wick component than the prototypes by the authors' company is currently registered in the United Kingdom. This device has been the subject of recently reported field trials in England at a poultry farm, a flour mill, and a seed warehouse and performed well in these initial evaluations [*19*].

Acknowledgments

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Steven R. Spaulding[1] *and William B. Jackson*[2]

Field Methodology for Evaluating Rodenticide Efficacy

REFERENCE: Spaulding, S. R. and Jackson, W. B., **"Field Methodology for Evaluating Rodenticide Efficacy,"** *Vertebrate Pest Control and Management Materials: Fourth Symposium, ASTM STP 817*, D. E. Kaukeinen, Ed., American Society for Testing and Materials, Philadelphia, 1983, pp. 183-198.

ABSTRACT: Methodology utilized to evaluate the efficacy of the experimental rodenticide bromethalin is described. Test site selection and determination of appropriate census techniques for use in different situations were important to the success of the field trial program. The testing program was divided into the following phases: (1) familiarization period, (2) pretreatment census, (3) pretreatment lag phase, (4) treatment period, (5) posttreatment lag phase, (6) posttreatment census, and (7) snap-trapping. The computerized data handling system used for storage and retrieval of data generated in the field trial program is described. A combination of two or three of the following census techniques was used to evaluate rodent activity before and after toxicant treatment: (1) census feed consumption, (2) tracking activity, (3) burrow activity, (4) dropping counts, (5) live trapping, and (6) water consumption. Overall, percentage reductions in activity values were comparable among census techniques. Tracking activity values were lower than those of other techniques in several house mouse trials, indicating the sensitivity of this method for monitoring mouse activity. A representative Norway rat field trial is presented to illustrate data generated through utilization of the previously described methodology and testing program.

KEY WORDS: methodology, rodenticide, efficacy, commensal rodent, census technique, field trials, bromethalin, vertebrate pest control

Appropriate experimental design and methodology are crucial in studies designed to determine rodenticide efficacy. These determinations are rarely easy, since commensal rodent populations are dynamic, mobile, and variable, depending upon habitat and food sources. Rodenticide efficacy is determined by utilizing population census or index techniques before and after toxicant treatment and noting the subsequent reduction in rodent numbers or activity, or both.

[1]Biologist, Lilly Research Laboratories, Greenfield, Ind. 46140.
[2]Director, Center for Environmental Research and Services, Bowling Green State University, Bowling Green, Ohio 43403.

A field trial program designed to determine the efficacy of the experimental rodenticide bromethalin [*1,2*] was initiated in 1980. Subsequently, eight Norway rat (*Rattus norvegicus*) and nine house mouse (*Mus musculus*) trials were conducted in five geographical regions of the United States. Studies were classified as either indoor or outdoor based upon the location of the target rodent population's food source. Results were considered acceptable when at least two census techniques indicated a 70% or greater reduction in target population activity and a three-day posttest snap-trapping period resulted in trapping no more than one target animal per ten traps per night. The objective of this paper is to describe the methodology utilized in this rodenticide evaluation program and to discuss the census and activity techniques used, including those previously described by Peardon [*3*] and Kaukeinen [*4*].

Test Site Selection

Selection of suitable test sites was particularly important for developing test data within the limitations of the experimental sequence, materials, and manpower. A rodent population of 25 to 200 was selected because it was of sufficient size to evaluate the efficacy of the rodenticide but not so large as to require excessive manpower and equipment. Preliminary evaluation of rodent signs such as droppings, burrows, runways, and feeding sites contributed to determination of rodent species and relative population size. Observations by property owners and employees also were useful in determining the extent of the rodent infestation.

Efforts were made to locate isolated populations, since rodents from peripheral populations may rapidly invade or reinfest treated areas due to the void created by poisoning. Potential trial sites near other infested structures or sites which were part of a larger structure or complex (such as stockyards, large warehouses, or multiple block urban tracts) were avoided.

The investigators obtained the cooperation of the property owner and employees and thoroughly explained the nature of the toxicant and test program. The importance of preventing equipment and bait disturbance or making significant changes in the test site was emphasized. Test sites were selected where relevant local, state, and federal regulations could be met.

Safety was a major consideration in selecting sites and conducting field trials. Cautious baiting procedures minimized the chances of contaminating foodstuffs, animal feed, water, and the environment or of creating other hazards to humans and other nontarget species. The sites were free of disturbance by uncontrolled pets and children.

Several other factors were considered when selecting test sites. The presence of only one rodent species simplified the census and evaluation; however, mixed infestations are common and were utilized in several trials. A test site must not have received chemical rodent control for at least one

month prior to initiation of a trial. Sites with an abundance of highly preferred foods were avoided where possible. Finally, the site and test period were chosen to minimize disruptive effects of adverse weather.

Testing Program

The testing program to evaluate the efficacy of bromethalin was divided into the following periods: (1) familiarization, (2) pretreatment census, (3) pretreatment lag phase, (4) treatment, (5) posttreatment lag phase, (6) posttreatment census, and (7) snap-trapping.

A familiarization period of several days permitted verification of the rodent species present by live trapping, tracking board observations, and searching for rodent signs; determining appropriate census techniques; locating areas of greatest rodent activity; and testing alternative census baits. The pretreatment and posttreatment censuses usually lasted three days but were extended to four days in several trials using live trapping. A seven-day census period was necessary before feed consumption stabilized in two mouse trials. Site maps were prepared indicating census and toxicant baiting points, snap-trap and carcass recovery locations, and physical characteristics of the trial site; photographs also were useful for future reference. A lag phase of three days with no disturbance immediately followed the pretreatment census and treatment phase to reduce prebaiting effects. A three-day snap-trapping period immediately followed the posttreatment census. Approximately equal numbers of snap-traps and toxic baiting points were utilized.

During the treatment phase, bromethalin meal bait was distributed in bait stations or placed directly in burrows in preweighed packages. Bait stations were placed at intervals of 3 to 7 m (10 to 20 ft) for rats and 1.5 to 3 m (5 to 10 ft) for mice in areas where rodents were most likely to find and consume the bait (that is, along walls, in corners, and in other concealed locations). Toxic bait was placed in open containers in several trial sites which were inaccessible to nontarget species. The toxicant was available to rodents until feeding activity was minimal or had ceased. Burrows were baited until they were inactive. When feed consumption was used as a census technique, toxic bait placements were in different locations from those of the census periods to avoid preconditioning. A moisture control station, similar to the one holding toxicant, was placed in an undisturbed location in the trial site to allow determinations of daily moisture loss or gain by the toxic bait. These stations were inaccessible to target and nontarget species.

In general, bromethalin consumption was high on Day 1 and had decreased dramatically by the second or third day. A second peak in consumption frequently appeared between Days 5 and 10, after which consumption again returned to a low level. In sites with complex environments, additional peaks in activity were occasionally observed. Representative treatment-phase

data for Norway rat and house mouse trials illustrating this second peak in toxicant consumption are presented in Figs. 1 and 2, respectively. These waves or peaks in feeding activity were probably related to the social behavior of rodents; animals receiving a sublethal dose, recovering, and coming back to feed; or invasion by animals from peripheral habitats. Under natural conditions, feeding by socially inferior rodents may be inhibited by dominant animals in the population. Following poisoning, the inferior rodents' feeding from bait stations could result in a second bait consumption peak. It was im-

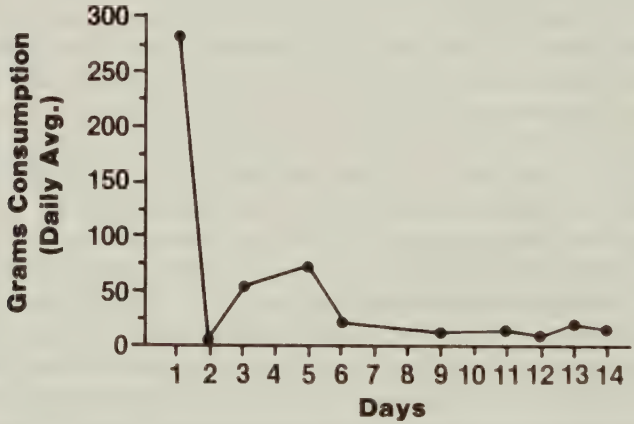

FIG. 1—*Bromethalin bait consumption observed from a Norway rat population on a Kansas hog farm.*

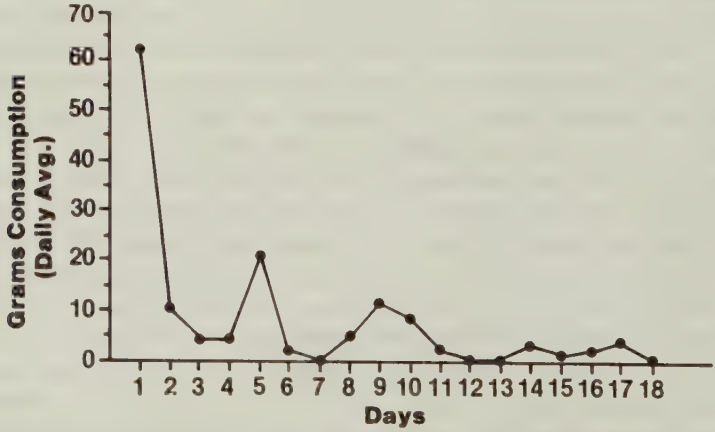

FIG. 2—*Bromethalin bait consumption observed from a house mouse population in a Pennsylvania feed storage room.*

portant that these patterns be recognized so that the treatment period was not prematurely terminated with the false impression of product failure.

A program of perimeter baiting, to prevent rodent invasion from surrounding areas, often was essential to the success of the field trial program. Areas adjacent to trial sites were carefully inspected for the presence of rodents and baited heavily with bromethalin bait from the initiation of the treatment phase until termination of the trial. Carcass recoveries and amount of toxicant distributed to these perimeter areas were recorded daily.

Computerized Data Handling System

Data generated in the field trial program were entered into a computerized data base system (RAMIS). A representative completed daily observation data form and instructions are presented in Figs. 3*a* and 3*b*, respectively. This form was used to record census bait/toxicant consumption, tracking and burrow activity, dropping counts, and water consumption. Another similar form was used to record live trapping and carcass recovery information.

The advantage of utilizing this computerized data handling system was the efficient storage and retrieval of a relatively large volume of data and the subsequent generation of printouts summarizing trial results. First the data were keypunched when duplicate copies of data forms were received from investigators in the field; printouts of raw data were requested immediately for verification. Upon verification, final summary tables were requested in such a format that they could be used to meet data submission requirements of regulatory agencies (see Fig. 4).

The primary disadvantage of the system was inflexibility. For example, computer consumption calculations could be made only if all the bait stations contained equal amounts of toxicant/census feed. It was realized early in the trial program that modifications to the system would allow greater flexibility in amounts of toxicant/census feed placed in bait stations based upon rodent activity levels at a particular location. This modification could be easily made with a minor change in the program. Overall, it was felt that the benefits and efficiency of the computer data system outweighed the disadvantages, particularly when the tremendous savings in man-days related to data handling was considered.

Census Techniques

Selection of appropriate census or activity index techniques suited to conditions of each trial site was crucial in developing successful trial data. Generally, three procedures established during the familiarization period were selected from the following: (1) census feed consumption, (2) tracking activity, (3) burrow activity, (4) dropping counts, (5) live trapping, and (6) water consumption. Overall, the census technique values were more com-

TRIAL LOCATION: RALEIGH, N.C. LIVESTOCK BARN

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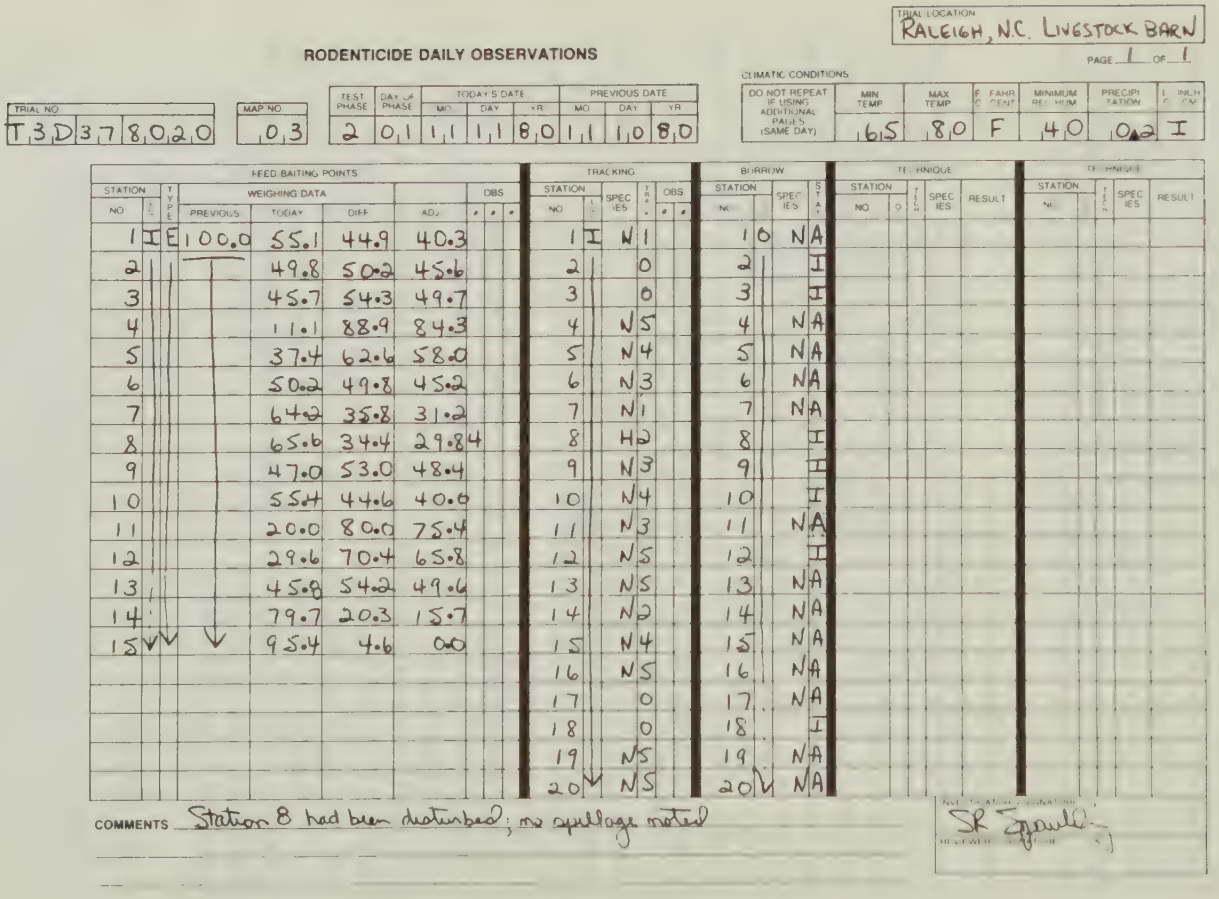

RODENTICIDE DAILY OBSERVATIONS

TRIAL NO	MAP NO	TEST PHASE	DAY OF PHASE	TODAY'S DATE MO	TODAY'S DATE DAY	TODAY'S DATE YR	PREVIOUS DATE MO	PREVIOUS DATE DAY	PREVIOUS DATE YR
T3D378020	03	2	01	11	11	80	11	10	80

CLIMATIC CONDITIONS

DO NOT REPEAT IF USING ADDITIONAL PAGES (SAME DAY)	MIN TEMP	MAX TEMP	F C FAHR CENT	MINIMUM REL HUM	PRECIPITATION	L C INCH CM
	65	80	F	40	0.2	I

FEED BAITING POINTS

STATION NO		TYPE	WEIGHING DATA PREVIOUS	TODAY	DIFF	ADJ	OBS		
1	I	E	100.0	55.1	44.9	40.3			
2				49.8	50.2	45.6			
3				45.7	54.3	49.7			
4				11.1	88.9	84.3			
5				37.4	62.6	58.0			
6				50.2	49.8	45.2			
7				64.2	35.8	31.2			
8				65.6	34.4	29.84			
9				47.0	53.0	48.4			
10				55.4	44.6	40.0			
11				20.0	80.0	75.4			
12				29.6	70.4	65.8			
13				45.8	54.2	49.6			
14				79.7	20.3	15.7			
15				95.4	4.6	0.0			

TRACKING

STATION NO		SPECIES	TRAK	OBS		
1	I	N	1			
2			0			
3			0			
4		N	5			
5		N	4			
6		N	3			
7		N	1			
8		H	2			
9		N	3			
10		N	4			
11		N	3			
12		N	5			
13		N	5			
14		N	2			
15		N	4			
16		N	5			
17			0			
18			0			
19		N	5			
20		N	5			

BURROW

STATION NO		SPECIES	STAT
1	O	N	A
2			I
3			I
4		N	A
5		N	A
6		N	A
7		N	A
8			I
9			I
10			I
11		N	A
12			I
13		N	A
14		N	A
15		N	A
16		N	A
17		N	A
18			I
19		N	A
20		N	A

TECHNIQUE

STATION NO		TECH	SPECIES	RESULT

TECHNIQUE

STATION NO		TECH	SPECIES	RESULT

COMMENTS: Station 8 had been disturbed; no spillage noted

INVESTIGATOR'S SIGNATURE: SR Spaulding

HELPER'S SIGNATURE:

FIG. 3—(a) *Rodenticide daily observation data form.*

DAILY RAW DATA INSTRUCTIONS

Trial No – A Lilly assigned 9-digit experiment number, format described in the Animal Science Data Handling Manual

Map No – This number should correspond EXACTLY to the site map reference for this phase. REMINDER — Each phase requires a map

Trial Location – Indicate name of city, business or farm. If more than 1 site exists at a location, designate a number. For example, Greenfield 1, Greenfield 2

Climatic Cond – Obtain indicated information from HYGROTHERMAGRAPH

Test Phase – Fill an appropriate number
1 Familiarization
2 Pre-treatment Census
3 Treatment
4 Post-treatment Census

Day of Phase – Fill in the appropriate day of the phase for which data is being collected. Example 1, 2, 3, etc

Previous Date – Date of previous observation within test phase

Station No – Number of the census point or Rodenticide station. Should correspond EXACTLY to map

Location – I - Inside
O Outside

Type – O - Open Feed Bait Station
E Enclosed Feed Bait Station
C Control

Baiting Points – Record under "Previous" the weight to which feed baiting points were increased on the previous day. This will be a standard weight for each trial, and may be entered as some amount of feed bait (e.g. 200), or as some amount of feed bait plus a tare weight (e.g. 268). Also record the weight of the feed bait at each station for the current day under "Today". As in "Previous" values, you may record the weight of the feed bait alone, or the weight of the feed bait plus the tare weight. Be consistent. If you ever record weights which include tare weight, you must **always** record weight including tare weight. Also calculate difference between "Previous" and "Today" and record in "Diff". Optionally, you may calculate weight adjusted for moisture ("ADJ").

Observations – Fill in corresponding number, up to 3 observations.
1 – SPILLAGE — Attempt to reclaim and weigh as much spillage as possible.
2 FRESH FECES — Note when they are found in or near feed bait
3 WET FEED BAIT — Attempt to dry as much as possible before weighing
4 DISTURBANCE Note any disturbance of census points
5 CONTAMINATION By debris, etc
6 DEAD ANIMAL In feed bait station

Tracking Data

Species – Fill in the appropriate number, up to 2 observations of the species making the tracks
H House Mouse
N Norway Rat
R Roof Rat
NOTE — If more than one species sign appears at a single census point, repeat station number

Tracks – Fill in the number of tracks using the following key

0	0	3	11-20
1	1-5	4	21-30
2	6-10	5	31+

Observations – Refer to previous observations key. Only 4 and or 5 are valid

Burrow Data

Species – Refer to previous species key

Burrow Status – Record status of the burrow using the following key
A Active Burrow
I Burrow Inactive
N New Active Burrow

Other Techniques

Techniques – Indicate census technique using the following key
1 - Quantity of Feces
2 – Counting Rodents
3 Gnawing Other Damage
4 - Urine Detection
5 - Rubmarks — Runways
6 Water Consumption
NOTE — If more than one species sign appears at a single census point, repeat station number

Species – Refer to previous species key.

Result – Record the appropriate result
EXAMPLE. Number of feces, rubmarks, etc

Comments – Record any additional comments.

FIG. 3—(b) *Rodenticide daily observation data form instructions.*

CENSUS BAIT CONSUMPTION
TRIAL: T3D378020

TABLE 1

TEST-PHASE	DAY	DATE	TOTAL NO. STATIONS	NO. ACTIVE STATIONS	TOTAL BAIT CONSUMPTION	AVG.CONS. (G)/ ACTIVE STATION
PRE-TREATMENT	1	11/11/80	14	14	679.0	48.5
	2	11/12/80	14	14	613.2	43.8
	3	11/13/80	14	14	700.1	50.0
AVG. PRE-TREATMENT			14	14	664.1	47.4
POST-TREATMENT	1	12/05/80	14	13	10.0	0.8
	2	12/06/80	14	1	0.1	0.1
	3	12/07/80	14	2	0.3	0.2
AVG. POST-TREATMENT			14	5	3.5	0.3
		PERCENT REDUCTION		61.90	99.48	99.28

FIG. 4—*Summary of census bait consumption from a Norway rat rodenticide trial in a North Carolina livestock barn.*

parable within a trial for Norway rats than for house mice (Figs. 5 and 6). Discrepancies among census technique values will be discussed in the following paragraphs.

Census Feed Consumption

Census feed consumption traditionally has been used to evaluate rodenticide efficacy by providing a relative index of the size of the sampled rodent population [*3-6*]. This technique was utilized in the trial program in 16 of 17 trials. Livestock feed was presented as the census bait for three or four days (seven days in two mouse trials) before and after toxic bait treatment; the resulting change in consumption provided an estimate of the rodent population. Equal amounts of census feed (100 to 200 g for rats and 20 to 50 g for mice) were distributed in metal or plastic bait stations at intervals of approximately 3 m (10 ft) for mice and 7 m (20 ft) for rats, or as rodent signs or human or animal activity directed.

In order for feed consumption to provide a reliable index of rodent activity, acceptance of the census feed must be reasonably consistent and of measurable quantity. Active rodent feeding locations should be recognized during the familiarization period, so that bait stations with little or no consumption may be relocated to more desirable locations before initiation of the pretreatment census. Insufficient and inconsistent consumption of census feed was encountered with Norway rats (even though the toxicant was readily consumed) in a Pennsylvania dairy farm trial because of the abundance of competitive feed. Here, dropping counts provided an additional and suitable technique to estimate rat activity (Fig. 5).

Moisture control stations provided data that enabled computer adjustments for moisture gain or loss of census feed or toxicant. Ideally, a single control station reflected conditions throughout the entire trial site; however, this was found to apply only in situations with constant internal environments. Feed or toxicant consumption was difficult to quantify in sites with highly variable moisture conditions, since only moisture adjustments on stations near the control were reliable. Norway rat consumption values were "usable" since sufficient amounts of feed were consumed to overcome bias due to moisture variations between stations. However, mouse consumption was difficult to distinguish from moisture variations in the bait, since a few mice eat very little in a 24-h period. Thus, less confidence was placed in the accuracy of feed consumption as a census technique for the house mouse, especially in sites with variable internal moisture conditions (such as the Rhode Island feed mill and Pennsylvania feed storage room trials).

An additional issue related to bait stations and moisture controls involves their use in outdoor trials. Several days of consumption data were lost in outdoor trials in Indiana, Kansas, and Ohio due to precipitation or high winds, or both. Therefore, it was particularly useful to have employed at least three

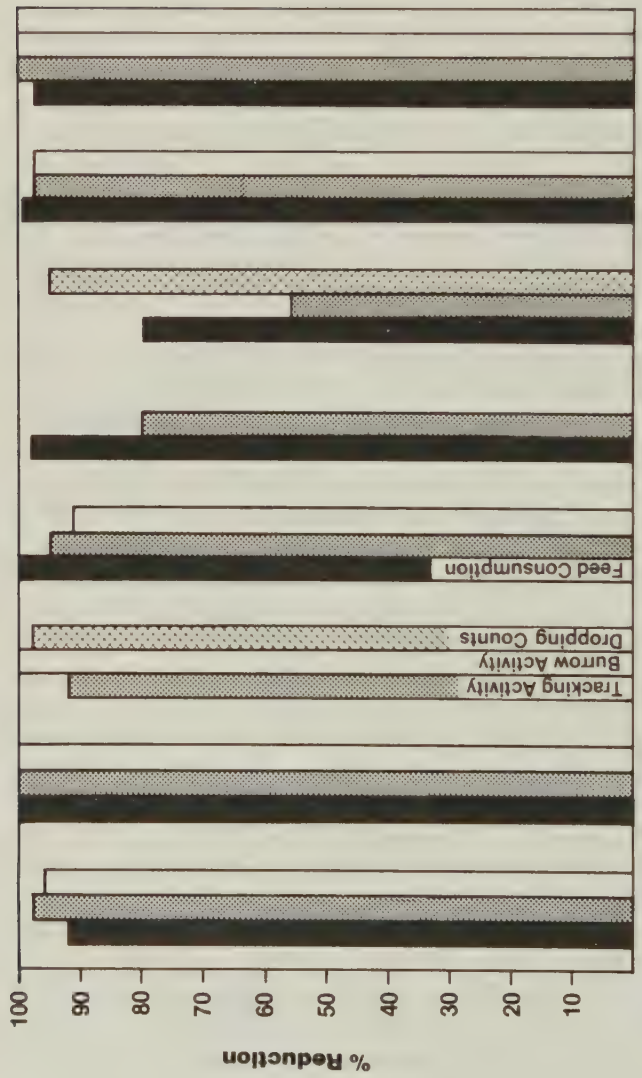

FIG. 5—*Percentage reduction in Norway rat activity following treatment with 0.005% bromethalin.*

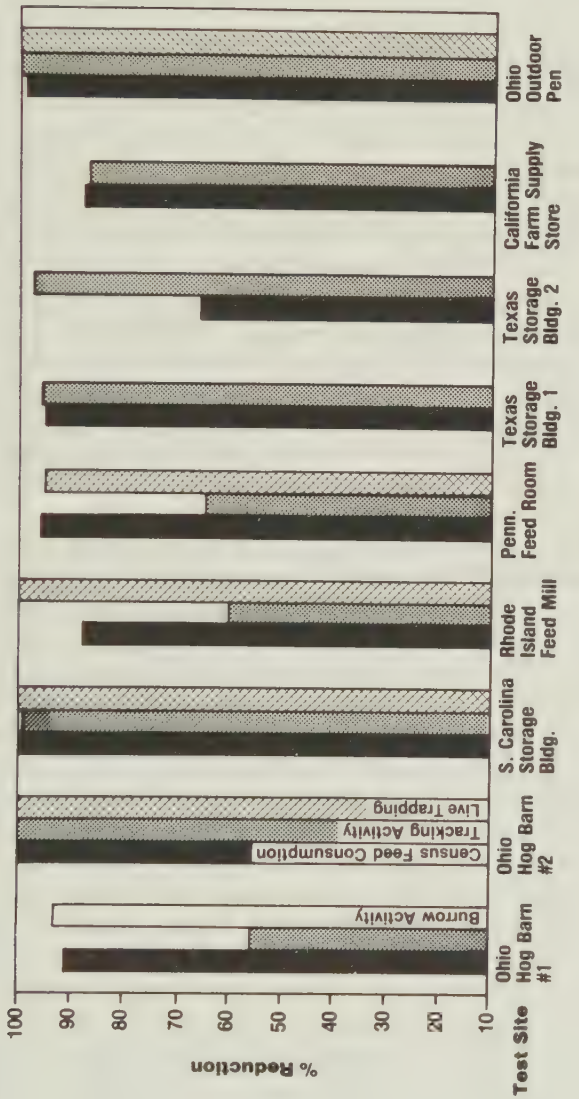

FIG. 6—*Percentage reduction in house mouse activity following treatment with 0.005% bromethalin.*

census techniques in outdoor trials to allow for loss of consumption data during inclement weather.

Tracking Activity

Tracking activity was utilized as a census technique in all the field trials and followed the methodology developed by Kaukeinen [*4*]. Precut sections of vinyl floor tile 8 by 15 cm (3 by 6 in.) were sprayed with a small, pressurized sprayer (Chromist, Fisher Scientific Co.) containing a chalk/acetone or alcohol mixture. Once applied, the solvent evaporated, leaving a thin deposit of chalk in which tracks were counted. In exposed environments, tracking boards were protected by placement inside bait stations or other available containers. However, tracking boards were never placed in bait stations containing census feed, since the data sets would not then have been independent.

Tracking boards were rated according to six classifications: 0 tracks, 1 to 5 tracks, 6 to 10 tracks, 11 to 20 tracks, 21 to 30 tracks, and over 30 tracks. Boards totally wiped clean by extensive rodent activity were classified as over 30 tracks. This method worked well in most cases; however, in high-density populations where many boards were totally cleaned in the pretreatment census, tracking activity in the posttreatment census (rated on the same scale) underestimated the actual reduction in activity.

An alternative, more traditional method to obtain rodent activity data involved the use of powdered limestone tracking patches placed directly on a given surface. The percentage of surface area tracked was determined to estimate rodent activity. In the trial at the California duck farm, tracking patches enabled data collection when rats became shy of tracking boards and bait stations.

Tracking activity was a reliable way to monitor both Norway rat and house mouse activity in most situations. Tracking activity at the Texas feed mill trial deviated most from other census technique values in determining Norway rat activity (Fig. 5). Here, tracking activity was reduced only 54% compared to 80% for feed consumption and 95% for dropping counts. Active reinfestation of the feed mill from adjacent feed bunks was monitored through placement of tracking boards near entry points. The disparity in census values may have resulted from placing tracking boards too close together at key entry points and therefore monitoring the activity of only a few rats on several different boards.

In house mouse trials, the percentage reduction in house mouse activity measured by tracking boards was not consistent with other census technique values in Ohio, Rhode Island, and Pennsylvania trials (Fig. 6). This phenomenon was attributed to behavior patterns observed in mouse populations. Generally, mice have relatively small home ranges, and distributing toxicant to those individuals in inaccessible areas (such as ceilings, walls, behind

stored materials) often is impossible. Thus, as individuals in accessible locations are poisoned, they are replaced by those from inaccessible areas expanding their home ranges and contacting the tracking boards for the first time. Therefore, if tracking boards have been placed in key locations, such as near food sources, a few mice from inaccessible areas remaining at the end of the trial are likely to produce an inflated activity index.

Utilization of tracking boards in mixed infestations enabled indirect observations of complex interspecific relationships that otherwise might go unnoticed, since it was possible to distinguish between rat and mouse tracks on the boards. While mixed infestations generally were avoided in the trial program, several situations were encountered in which Norway rats were the dominant species; and house mice were present at much lower population levels. This relationship, often not apparent until the end of the trial, was related to interactions between the two species. In undisturbed situations, rats suppress the movement patterns of mice and limit their access to food. As a result, the mouse population, often in inaccessible places, was difficult to census through tracking board observations. As rats began dying after feeding on the toxicant, the mice expanded their home ranges and contacted tracking boards for the first time; extended treatment periods were necessary to control such mixed infestations.

Burrow Activity

Monitoring active burrows for measuring rodent abundance and activity has been useful in agricultural areas [*7*] and urban environments [*8*]. Burrow activity was used successfully in six Norway rat trials and one house mouse trial. The procedure involved closing all active burrows with soil and then recording new and reopened burrows for the same length of time before and after toxicant treatment.

Burrow activity values were comparable with other census techniques in Norway rat and house mouse field trials (Figs. 5 and 6). This method was particularly valuable in trials at livestock facilities, since bait stations and tracking boards often were disturbed. In addition, data were not lost in outdoor trials due to inclement weather, since burrow status was not affected by precipitation or winds.

Dropping Counts

Dropping counts were utilized as a measure of activity in three Norway rat trials. Areas of high-density rodent droppings were located, daily counts taken, and the areas then swept clean. The most difficult aspect in successfully utilizing this method was determining the areas where droppings were consistently found, which often required several days during the familiarization period. This technique was particularly useful where disturbance of census points by humans or animals was likely to occur.

Live Trapping

Live trapping was utilized in one Norway rat trial and five house mouse trials. Tomahawk live traps (12.7 by 12.7 by 40.6 cm) were distributed at approximately 7-m (20-ft) intervals for rats, and Sherman live traps (7.6 by 7.6 by 22.9 cm) were placed at approximately 3-m (10-ft) intervals for mice. The traps were baited with peanut butter and checked daily at about the same time. The rodents were toe-clipped; information on trap location, sex, animal number, weight, and reproductive condition was recorded; and they were released at the point of capture.

Live trapping, marking, and releasing was the most informative of all census techniques, since the investigator was actually estimating the number of rodents in the trial site; and valuable research information regarding sex ratios, age structure, movement patterns, and the reproductive condition of the rodent population was obtained. However, live trapping was too time-consuming to be practical for other than special research purposes, and experienced investigators were essential if Norway rats were to be handled and marked properly. The reduction in activity values determined by live trapping were comparable to those obtained by other techniques in the field trials (Figs. 5 and 6).

Water Consumption

Water consumption by rodents before and after toxicant treatment has potential as a census technique in sites with limited water and was utilized to monitor mouse activity in one trial. Chick-waterers, filled with 200 mL of water, were placed in locations where the greatest potential water intake had been determined previously. One waterer was placed inside a wire mesh cage to serve as a control to estimate water loss from evaporation.

Potential problems with this methodology include (1) consumption by nontarget species, (2) additional water sources in the trial site, and (3) frequent failure of control stations to represent variable evaporation rates throughout the site, which may reduce the reliability of consumption values. The latter condition was present in the house mouse trial in a farm supply store in California (Table 1) and therefore accounted for a considerably lower value for reduction in activity determined by water consumption (39%) when compared with more consistent reduction in activity values for feed consumption (88%) and tracking activity (88%).

Representative Field Trial

Representative data from an indoor Norway rat bromethalin field trial are presented in Fig. 7. Census feed consumption, tracking activity, and burrow activity all indicate nearly complete elimination of the Norway rat popula-

TABLE 1—*Summary of water consumption from a house mouse rodenticide trial in a California farm supply store.*

	Month/Day of Water Intake, mL					
	Pretreatment			Posttreatment		
Station No.	7/15	7/16	7/17	8/12	8/13	8/14
2	28	97	64	23	19	11
3	15	14	10	34	27	10
4	24	58	67	37	38	0
5	21	25	20	14	44	41
6	29	22	19	47	22	17
7	23	21	18	15	20	43
8	39	73	67	29	24	20
Total	179	310	265	199	194	142
Control	13	12	13	12	13	9
Adjusted total	88	226	174	115	103	49
Daily average, mL		23.2			14.1	
Adjusted percent reduction in activity			39.1			

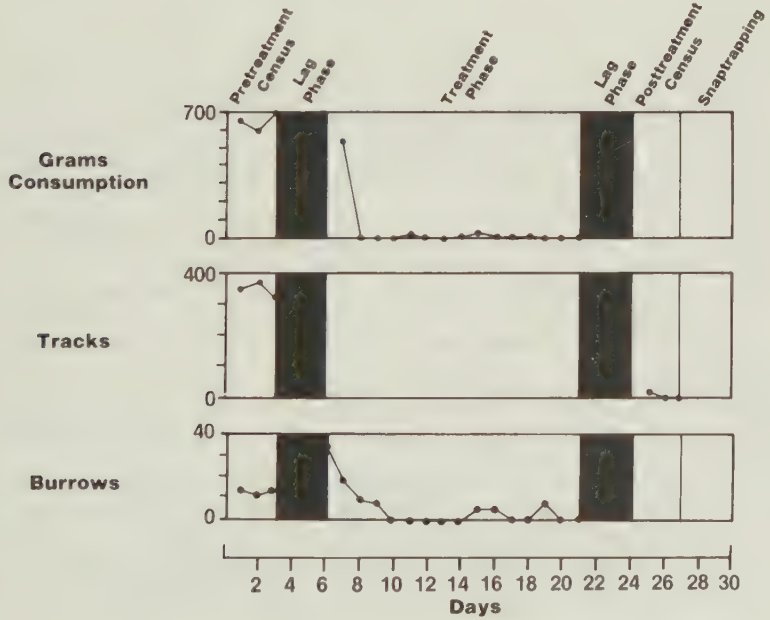

FIG. 7—*Representative bromethalin field trial data on a Norway rat infestation in a livestock barn in North Carolina.*

tion. Bromethalin was distributed in bait stations and in 30-g plastic packets placed in active burrows for a 15-day treatment period. The treatment period was extended to 15 days since burrow activity increased on Days 9 and 10. No rats were captured during the final snap-trapping phase.

Conclusions

Test site selection and determination of the appropriate census techniques to use in different situations were crucial to the success of this trial program. While most census techniques provided only an index to population size, reductions in activity generally were consistent among techniques and therefore gave good indications of rodenticide efficacy. The utilization of three census techniques in field trials provides backup in case unforeseen events should invalidate one of the procedures.

Acknowledgments

The authors greatly appreciate the efforts of the many contract investigators and Lilly field research representatives who were instrumental in the success of the field trial program. The assistance of Dr. A. L. Melliere in project guidance and manuscript review is also greatly appreciated.

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James F. Glahn[1] *and G. Keith LaVoie*[2]

An Approach for Evaluating Food Container Resistance to Damage by Individual Norway Rats

REFERENCE: Glahn, J. F. and LaVoie, G. K., **"An Approach for Evaluating Food Container Resistance to Damage by Individual Norway Rats,"** *Vertebrate Pest Control and Management Materials: Fourth Symposium, ASTM STP 817*, D. E. Kaukeinen, Ed., American Society for Testing and Materials, Philadelphia, 1983, pp. 199–208.

ABSTRACT: A test chamber incorporating a remotely triggered low-light level video camera and recorder is described for evaluating food container resistance to damage by Norway rats (*Rattus norvegicus*). Data presented on the actual gnawing times necessary for a rat to penetrate a standard container and behavioral patterns associated with penetration suggest that this approach is a reliable measure of container resistance. To account for variation in penetration times both within and among test animals, we present a simple damage resistance index for test containers using a three-day test series to compare penetration times of test containers with an average of the penetration times for two standard containers. The mean index values derived in this manner appear sufficiently sensitive and reliable to rate the relative resistance of test containers. Application of this testing apparatus and method is described, including its limitations and possible modifications.

KEY WORDS: vertebrate pest control, Norway rats (*Rattus norvegicus*), damage resistance, food containers

Rodents cause extremely large losses of packaged foodstuffs in storage and in transit [*1*]. In addition to direct losses by consumption, rodent damage to packaged food results in spillage and contamination of goods and affords easy entry to various spoilage organisms. Many species of rodents throughout the world may be involved in this damage problem; however, commensal rodents, in particular the Norway rat (*Rattus norvegicus*) and house mouse (*Mus musculus*), are the principal pest species. Although improved food

[1]Wildlife biologist, U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Kentucky Research Station, Bowling Green, Ky. 42101.

[2]Wildlife biologist, U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Denver, Colo. 80225.

handling and rodent control procedures could reduce these losses, in certain situations rodent-resistant packaging may be the only practical means of controlling damage.

Although research on Norway rat damage and control has been extensive, little of it has dealt with resistant packaging as a control method. A basic need in this area is a quantitative method to rate the physical resistance of potential materials and designs to rat damage. Two methods have been explored in the past for examining the resistance of materials. These include measuring the time between initial exposure and penetration in cage tests [*2*] and rat activity measured in an obstruction apparatus [*3,4*]. This latter method used a photocell-activated timing apparatus which recorded the time a rat spent in proximity to the test material and involved an extensive conditioning and fasting regimen.

Another possible approach for evaluating the resistance of package materials is measuring the actual time a rat spends gnawing on a test material until it is penetrated. We describe this approach for evaluating food container resistance utilizing a videotape apparatus.

Procedures

Testing Apparatus

The experimental chamber (Fig. 1) was constructed from a 1.8- by 0.6- by 0.6-m galvanized metal stock tank divided by a central sheet metal partition into a forward and rear compartment. In the rear compartment a harborage box and water dish were provided for the test animal. The forward compartment was further partitioned by a sheet metal barrier plate on which a 10-W red light source and digital clock were mounted. The food container to be evaluated was friction-held in a rectangular hole in the base of the barrier plate with about one third of the container exposed. Access through the central partition into the forward compartment was permitted only through an enclosed mercury switch treadle at the base of the partition. A hinged plywood cover enclosed the experimental chamber during testing and opened to service the forward and rear compartments. A recessed box in the center of the plywood cover, equipped with a forward facing plate glass window, held the low-light level video camera (JVC Model No. CTC-5000).[3] This camera, equipped with a 12.5-mm wide angle lens, allowed almost the entire forward compartment to be monitored under low-intensity red light. The camera output was to a videotape recorder (JVC Model No. FV-3500) having a 1-h videotape. The electrical circuit of the recorder was completed when the treadle was in the forward-down position, allowing animal activity to be

[3]Reference to trade names does not imply U.S. government endorsement of commercial products.

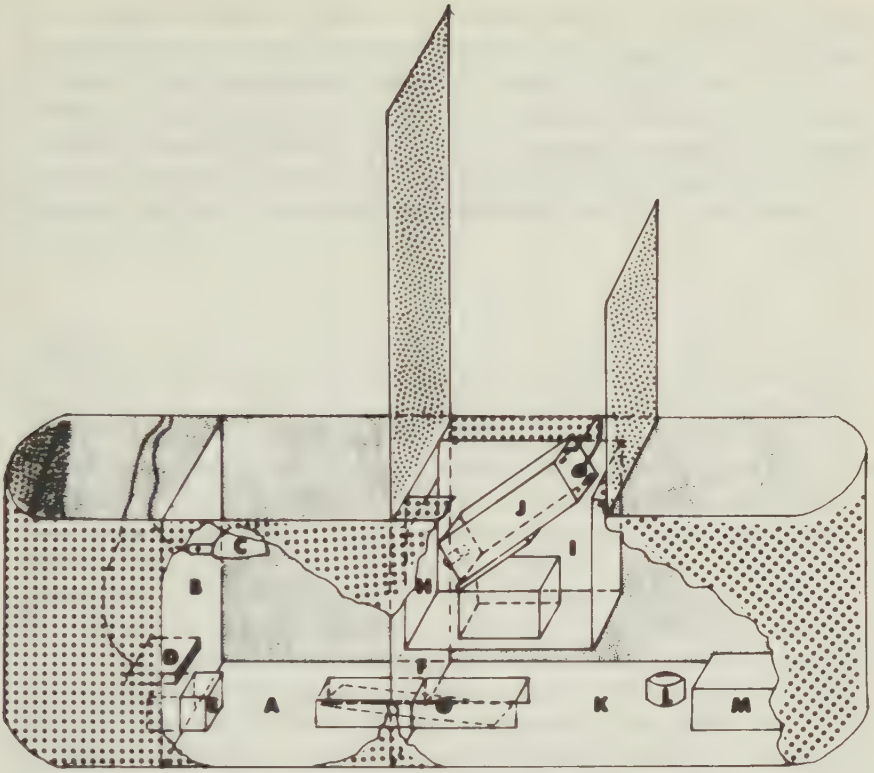

FIG. 1—*Experimental chamber used in container penetration trials, consisting of a forward test compartment* (A) *with a barrier plate* (B), *red light source* (C), *digital clock* (D), *and food container* (E); *a central partition* (F) *with a mercury switch treadle* (G), *plate glass window* (H), *camera box* (I), *and video camera* (J); *and a rear or harborage compartment* (K) *with a water dish* (L) *and harborage box* (M).

recorded when the animal was in the forward compartment. Activity was monitored on a JVC video monitor (Model No. 6201).

Test Animals and Preconditioning

Test animals were wild-trapped adult Norway rats held in captivity for a minimum of 30 days before testing. Animal selection was based on the consistency of an individual in penetrating standard 1.378-MPa (200-psi) bursting strength fiberboard containers (for example, a corrugated cardboard shipping box) during a single overnight (4 P.M. to 8 A.M.) exposure in at least three consecutive trials. Our initial cage trials indicated that few rats, even when food deprived, would spontaneously penetrate food containers. Conditioning rats in standard laboratory cages initially to penetrate containers involved inserting a rat chow pellet in a small hole in the corner of a

container and allowing the rat to enlarge the hole to obtain additional food found inside these containers. In this process, the rats learned to penetrate intact food containers in cage trials. Even after this training, some of the rats failed to meet the selection criteria and were discarded from further trials. Three rats were initially selected to examine behavioral patterns and variation in performance of container penetration. Six rats, including two from the former group, were used to examine procedures for rating food container resistance.

Test Procedures

Twenty-four hours before the start of each test series, one of the test animals was placed in the test chamber for acclimatization and food ration scheduling and given a ration of three laboratory chow pellets (about 20 g total). An empty standard 1.378-MPa (200-psi) container was positioned in the barrier plate to be explored but it contained no food. The rat remained in the test chamber and was fasted until the start of the test beginning between 4 and 5 P.M. of the following day. At the start of this test a new standard 1.378-MPa (200-psi) fiberboard container with three laboratory chow pellets taped to the rear inside wall was placed in the barrier plate, and a videotape was placed in the recorder to monitor activity. Activity recordings were produced at night when Norway rats are the most active and the disturbance from personnel working around the laboratory would be reduced. Since red light is poorly perceived by these rats, conditions simulated near darkness. This testing was repeated for two more consecutive evenings by using a test container and another standard container, respectively, in a single-choice three-day test series.

The video recording from each test night was viewed, and the length of rat activity in the forward compartment, as well as the gnawing time necessary for the rat to obtain the first food pellet from the container (penetration time), was timed with a stopwatch. For three rats the frequency of selected behavioral acts before, during, and after the sequence of container penetration were also recorded, as well as the duration of each observational period.

To account for variation in penetration times on standard containers and to rate test containers to a relative standard, we devised a simple damage index based on each three-day test series per replication. The index value, I, for a test container from one test series was computed from the following formula where Observations A and C are the penetration times on standard 1.378-MPa (200-psi) bursting strength containers from the first and third night, respectively, and Observation B is the penetration time on the test container

$$I = \frac{\mathrm{B}}{(\mathrm{A} + \mathrm{C})/2}$$

This index formula attempts to account for changes in animal learning and motivation between Trial A and Trial B by incorporating the animal's performance on Trial C. It assumes that the potential performance on Trial B is a function of the average performance on Trials A and C. Thus, index values of 1 would indicate no difference between the test and standard container, whereas values greater than 1 would indicate greater resistance of the test container. A t-test was used to examine for differences in the mean index values obtained.

Results

Behavioral Patterns of Container Penetration

The general behavioral sequence of container penetration of three test animals examined in 45 observations indicated the need for the videotape apparatus in making direct observations of damaging behavior and the possibility of errors from attempting to measure damaging activity with a simpler activity recording device. Before the first gnawing attack, a brief exploratory period, lasting a mean time of 2.3 min (standard error = 0.30) for the three rats tested, always occurred. During this period, exploratory behavior in direct proximity to the food container consisted of sniffing and mounting (placing forelegs on) the container and could be misinterpreted by an activity recorder as gnawing behavior. The actual penetration period began immediately after this exploratory period and was initiated with the first gnawing attack. The penetration time was always broken into several (from two to ten) distinct gnawing attacks. Thus, gnawing was steady but not incessant and was broken up by frequent pauses in which the rat stood momentarily next to the container without gnawing. During the penetration period, or the time between the first gnawing attack and completion by the rat of a large enough hole in the container to obtain the first food pellet, the actual penetration time constituted a variable amount of the rat's overall activity in the forward compartment, ranging from 37 to 97% for those rats examined.

Probably the most serious problem we observed that would add variation to data gathered with an automated activity recorder was extraneous gnawing attacks on the container after the food had been removed. Although normally these attacks ended almost immediately after the rat obtained the first food pellet, they did occasionally extend considerably beyond this point. Since these extraneous gnawing attacks probably have little relevance to container resistance, we excluded them from our measurement of penetration time. However, data on 45 observations of three rats suggest that this activity could represent up to 20% of the overall gnawing time for an observation with an average of about 12%.

Indices of Container Resistance

Table 1 summarizes the data on penetration times for the three rats tested a minimum of twelve times on standard 1.378-MPa (200-psi) bursting strength containers. The actual penetration times to obtain the first pellet ranged from 2.1 to 9.9 min, with an overall mean of 4.55 min (standard error = 0.26). A one-way analysis of variance indicated a significant difference ($P < 0.01$) among penetration times for individual rats, and the coefficient of variation for individual rats ranged from 22 to 40%. These data suggest a need to account for the variability in penetration times on standard containers with an averaging method such as the index previously described.

Table 2 presents the penetration times and resulting damage index values from seven completed test series on four individual rats in which the standard 1.378-MPa (200-psi) container was tested against itself to examine the reliability of the indexing procedure. Although the sample size is small, a *t*-test indicated a mean index value not significantly different from 1 ($P >$

TABLE 1—*Penetration times of three rats on standard 1.378-MPa (200-psi) bursting strength containers.*

Observation	Actual Penetration Time, min			
	Rat No. 1	Rat No. 2	Rat No. 3	Overall Summary
1	7.12	5.33	3.41	
2	7.87	7.82	3.31	
3	4.68	5.03	3.50	
4	2.08	3.93	2.41	
5	2.35	4.33	4.00	
6	3.78	3.25	5.20	
7	3.92	5.40	9.93	
8	2.85	6.05	7.18	
9	3.41	6.00	5.75	
10	3.75	5.62	5.90	
11	3.73	6.18	6.33	
12	3.78	6.33	5.78	
13	2.41	...	...	
14	2.92	...	...	
15	2.58	...	...	
16	2.73	...	...	
17	3.52	...	...	
18	3.27	...	...	
19	3.27	...	...	
20	3.31	...	...	
21	3.35	...	...	
$\bar{X}$	3.65	5.44	5.23	4.55
Standard error	0.31	0.35	0.60	0.26
Coefficient of variation, %	39.0	22.3	39.8	38.7

TABLE 2—*Damage index values derived from rat penetration times of standard 1.378-MPa (200-psi) bursting strength fiberboard containers (Observations A, B, and C).*

Rat No.	Penetration Times by Observation, min			Index Value
	A	B	C	
2	5.33	7.81	5.03	1.5
2	3.93	4.33	3.25	1.2
2	5.40	6.05	6.00	1.1
2	5.62	6.18	6.33	1.0
3	9.93	7.18	5.75	0.9
4	3.43	3.12	1.12	1.4
5	2.23	5.65	3.05	2.1
$\bar{X}$ (SE)[a]				1.3 (0.15)
$\bar{X}$ (SE)[b]				1.4 (0.26)

[a]Mean and standard error of index values, assuming all observations are independent ($N = 7$).

[b]Mean and standard error of index values used in t statistic treating the average value for Rat No. 2 as only one independent observation ($N = 4$).

0.20), even though considerable variation among penetration times existed both within and among individuals.

Table 3 presents the penetration times and resulting damage index values from six test series with four test animals comparing 2.756-MPa (400-psi) bursting strength containers (for example, a corrugated cardboard shipping box) with the standard to examine whether index values could detect such differences in container strength. A t-test to examine the differences in these two adjusted[4] index means indicated a significant difference ($t = -4.39$, $P < 0.01$) between the mean index values for 1.378-MPa (200-psi) and 2.756-MPa (400-psi) bursting strength containers.

Discussion

The camera angle provided a clear view of the rat's activity in proximity to the food container. However, the addition of sound recordings used in subsequent trials could increase the precision of measuring gnawing activity. The results indicate that the penetration time, or the actual gnawing time between the first attack on a container and the time the first pellet is removed, is a reliable index of container resistance. Although it would be desirable, from the standpoint of simplicity and of reducing subjectivity in the measurement of penetration time, to use some type of automated activity recorder to collect these data, certain inherent behavioral patterns in the act of container

[4]Means adjusted for repeated measures on the same test animal.

TABLE 3—*Damage index values derived from rat penetration times comparing 2.756-MPa (400-psi) (Observation B) with standard 1.378-MPa (200-psi) bursting strength containers (Observations A and C).*

Rat No.	Penetration Times by Observation, min			Index Value
	A	B	C	
4	3.10	19.20	2.30	7.1
5	4.67	16.53	2.30	4.7
6	4.40	16.70	6.73	3.0
6	7.05	30.43	5.33	4.9
6	12.01	17.58	7.38	1.8
7	3.07	13.38	1.47	5.9
$\bar{X}$ (SE)[a]				4.57 (0.78)
$\bar{X}$ (SE)[b]				5.23 (0.84)

[a]Mean and standard error of index value, assuming all observations are independent ($N = 6$).

[b]Mean and standard error of index values used in t statistic treating the average value for Rat No. 6 as only one independent observation ($N = 4$).

penetration may present problems by creating extraneous variation. Certainly, elaborate motivation control, as suggested by previous studies [*4*], may account for some but not all of this variation. However, measurements of rat activity in proximity to containers appear to be less reliable indicators of container resistance than actual gnawing time.

Although actual penetration times show considerable variation both within and among individuals, the methodology described, incorporating the indexing procedure which relates penetration times on test containers to the average penetration times on standard containers, appears to account for some of this variation. Thus, mean index values of standard containers approximated unity and yet were sensitive enough to show significant differences with stronger containers.

Although our data are limited, they appear to demonstrate the utility of this approach in obtaining an accurate relative measurement of container resistance by minimizing extraneous sources of variation by use of the remote observational technique discussed. Although a limited amount of preliminary training is necessary with this procedure, neither elaborate training nor motivation control scheduling, as suggested by previous studies [*4*], appears to be necessary. Actual gnawing times may be more directly a function of container resistance and, to a lesser degree, influenced by motivation and learning by the test animal. The indexing procedure tended to account for some of the variation due to animal learning and motivation over a particular test series sequence.

Although this approach has potential advantages in rating resistance of packaging material, it was found to have certain limitations. Long time periods are involved in evaluating an individual test material, with a single test series taking up to one week per replication. Equipment costs also limit the number of test chambers that could be used simultaneously. Further, we found that a 1-h videotape was not adequate to sufficiently record damage attacks on more resistant materials used in later trials. Thus, use of a recorder with a larger tape capacity would be necessary with more resistant packaging materials, and certain procedural changes would need to be incorporated if penetration required more than several days to complete. A possible consideration in this situation would be measuring the weight of material removed or surface area damaged per length of gnawing attack to reduce the amount of time required to conduct tests. In previously reported studies [5], we found that the amount of area penetrated over time of exposure could be related to container resistance when the materials compared were grossly different in physical properties.

In comparison with other methods, this penetration-time approach may provide a more sensitive means of evaluating the relative resistance of materials by rating this resistance on the relative amount of gnawing effort expended by individual rats to achieve penetration. Similar data based on container exposure time until penetration can be efficiently obtained in cage tests and may have more practical application in screening materials. However, since these exposure time data do not necessarily reflect effort by rats in achieving penetration, they may not detect differences in resistance among materials and designs which might need to be distinguished at some phase of packaging development. At this point, the videotape apparatus may also be useful in examining the details of rat gnawing behavior on various materials and designs under investigation.

Although this approach emphasizes controlling variation caused by intrinsic factors in container penetration, it does not consider the influence of certain extrinsic factors that could exist under field conditions. These include such things as social interaction or group response among rats, which might result in increased motivation among individuals during mutual attack on containers, and the availability of alternative food. Although these factors could significantly influence penetration time on containers under field conditions, they are unlikely to alter the rank ordering of container resistance ratings obtained with this approach. Thus, by comparing these relative values of container resistance in the laboratory, one or more of the most rodent-resistant designs could then be selected for field test evaluation.

Additional data are needed to examine the validity of the index rating procedure. However, it is hoped that the general approach and modifications suggested here may assist and encourage further studies of rodent-resistant packaging.

Acknowledgments

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Stephen C. Frantz[1] *and Constance M. Padula*[1]

A Laboratory Test Method for Evaluating the Efficacy of Glueboards for Trapping House Mice

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ABSTRACT: A laboratory test method developed for evaluating glueboard efficacy against house mice is described. The test method involves two different procedures for exposing mice to a particular glueboard product. The first procedure is to establish if mice captured by all four feet on glueboards will remain captured. During this 24-h study, individually caged mice are placed by hand on glueboards and observed at ten preset intervals for changes in glueboard holding ability and for changes in the health condition of captive mice. Positive results (regarding holding strength) from this screening technique leads to a second procedure to establish if "free-ranging" mice will go onto glueboards and be captured. In this case, the mice are tested as a social group in a 32-m^2 colony room where they make a choice of whether to enter a specially designed test apparatus (containing the food and water supply) via an open route or via a route containing a glueboard. Activity is monitored automatically, and food consumption is recorded daily over this twelve-day procedure (four days = pretest, four days = test, and four days = posttest), which allows social interaction and other behavioral factors to affect product efficacy. With the occasional appearance of new and modified glueboard traps on the market, a comparative basis for efficacy evaluation is desirable. Both testing procedures offered here yield useful information, though the first method could be simplified to ease data collection. The second procedure, by itself, may be sufficient for some purposes, and the results may be more applicable to field interpretation. Field application of glueboards, product design, and consumer considerations are discussed.

KEY WORDS: vertebrate pest control, glueboards, trapping, efficacy evaluation, mice

This study was designed to compare the efficacy of different glueboard products for capturing mice and to initiate discussion which might lead to the standardization of such laboratory procedures. Glueboards (glue traps,

[1]Rodent and bat specialist and assistant research scientist, respectively, New York State Department of Health, Center for Laboratories and Research, Laboratories for Virology, Empire State Plaza, Albany, N.Y. 12201.

sticky boards) may be defined as rodent control devices consisting of a flat, rigid base (such as cardboard, fiberboard, or plastic) coated on one side with an extremely adhesive material. The devices catch and hold rodents attempting to cross them, in much the same way as flypaper catches flies.

Although the concept of glueboards (sticky traps) is not new [*1,2*], it has only recently received popular attention [*3–5*]; recent issues of most pest control journals show several advertisements for these devices. The gain in popularity may be due to the various restrictions which have limited the use of some rodenticides and to a lack of efficacy with other available materials. Lacking in the sparse literature regarding glueboards are guidelines for assessing their efficacy. In essence the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) does not give the U.S. Environmental Protection Agency (EPA) authority to regulate pest control devices—those agents which use physical or mechanical means to trap, destroy, repel, or mitigate a pest—unless there is evidence that false or misleading statements have been made regarding a product [*6*]. To date, the EPA does not require the registration of glueboard products, and efficacy testing procedures and standards have not been developed by the EPA or the pest-control industry.

Two procedures for exposing mice to glueboards are described (a no-choice and a choice test) in order to answer three basic questions and thus provide criteria for comparing different products:

1. Will mice captured on glueboards remain captured for at least 24 h?
2. Will mice die during 24 h of captivity on glueboards?
3. Will free-ranging mice go onto glueboards and be captured?

Additional data were collected in order to provide some indication of the humaneness of glueboards. To provide a humane death, a product should render an animal unconscious and insensitive to pain as rapidly as possible, though the time until death may be delayed [*7*].

Materials and Methods

Test Animals

All the mice used in this study were laboratory-bred, ICR strain, albino house mice (*Mus musculus*). They were healthy, active, sexually mature, and, within seven days prior to the start of the test, weighed 15 to 35 g (average weight difference between sexes ≤ 5 g). For ectoparasite control, pyrethrum dust was applied to the animals not less than two days before the study was initiated.

Apparatus

No-Choice—Each mouse was kept in a separate screen-bottomed/front metal mouse cage having a floor surface area of 432 cm^2 (18 by 24 by 17 cm

high); this allowed for approximately 75% free space in the presence of a glueboard. Sheet metal pieces (10 by 14 cm) were bent at 45° angles along the middle of the long side to form A-framed shelters (6 cm high at the center). One shelter was used per cage during acclimatization, but only for control animals during testing.

Choice—Experimental mice were released into a mouse-proofed colony room (4 by 8 by 2.5 m), which allowed animals free access to any part of the room (see Fig. 1). Eighteen nest boxes (same cages as in the no-choice test) containing nesting material (shredded paper) were mounted on the room walls at various heights and were provided with at least one access route.

The center of the room contained a specially designed choice-apparatus constructed of two concentric walls of sheet metal (see Fig. 1). The outer wall was 1.5 m in diameter and the inner was 1.3 m in diameter; both walls were 0.6 m in height. The area within the inner wall was accessible to the mice via four tunnels (numbered 1 through 4) constructed of polyvinyl chloride (PVC) tubing (4 cm inside diameter by 20 cm length) which passed through the two walls at floor level and equidistant around the apparatus' circumference.

The inside (inner wall) entrance of each tunnel was fitted with a passive infrared motion detector and counter (Actimeter system, as described by Kaukeinen [*8*] and shown in Fig. 2) in order to continuously monitor activity, that is, the number of times an animal passes through the tunnel. Both body heat and motion are required to activate a count; thus, a mouse sitting in the viewing path of the Actimeter is not counted until it leaves the field of view—it

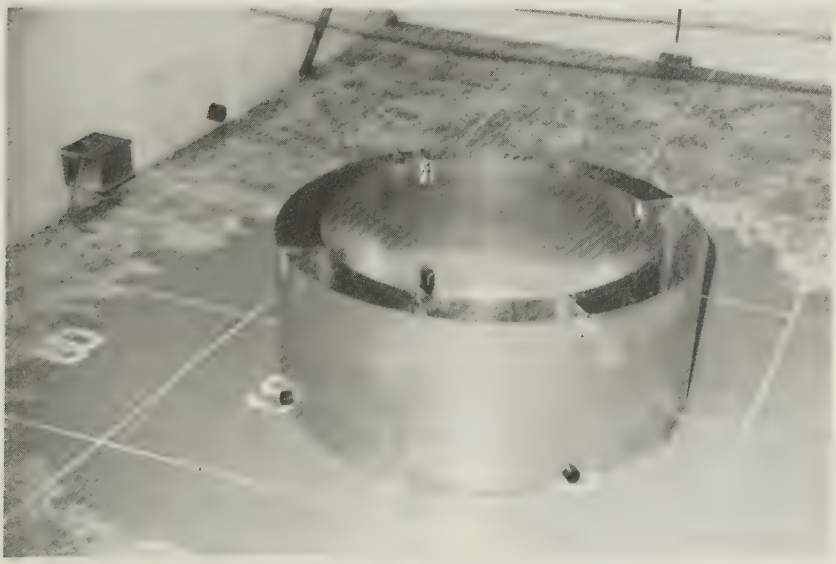

FIG. 1—*Choice test apparatus in mouse colony room.*

FIG. 2—*Mice captured on a glueboard (moved) near the tunnel entrance equipped with an activity counter.*

will be counted only once. Undoubtedly, miscounts are possible, but the Actimeters do provide a relative measure of activity.

The inner circle of the choice-apparatus contained the food and water supply as incentives for mice to pass through the tunnels, the only access routes. Both the metal feeders and glass water dispensers were designed so mice could not nestle or wallow in them.

For most of the choice test, the reference mice were released into screen-bottomed/front metal mouse cages having a floor surface area of 1536 cm^2 (64 by 24 by 17 cm high). A-framed shelters were provided, three per cage.

Glueboards

Although the study was not intended to be a product comparison, we worked with the following two glueboards in developing our procedures:

1. Stick-Em mouse glue traps
   Manufacturer: J. T. Eaton & Co., Inc., Twinsburg, Ohio
   Glue surface: 10.0 by 6.8 cm
   Total board dimensions: 11.5 by 8.5 by 1.0 cm
2. Trap-Stik glue traps
   Manufacturer: Southern Mill Creek Products Co., Inc., Tampa, Fla.
   Glue surface: 11.4 by 6.3 cm
   Total board dimensions: 12.7 by 8.3 by 1.0 cm

The two glueboard designs were similar in terms of adhesive surface dimensions, and both utilized a plastic tray for the rigid base material. A replicate of the current study using wild house mice would be necessary for a product comparison *per se*.

General Laboratory Conditions

The daily minimum/maximum air temperature of testing rooms averaged 25/29°C (range: 23 to 33°C) with an average relative humidity of 62% (range: 54 to 67%); the mice were not subjected to strong air currents. The testing rooms received 12 h of artificial light per day (6 a.m. to 6 p.m.), and the intensity of illumination did not exceed 700 lx (65 footcandles) at the closest location of mice to the light source.

No-Choice Procedure

Acclimatization—All the experimental and control mice (20 per group; sexes equal) were held under test conditions for at least two days before the test was initiated. The animals were not fasted or unduly stressed prior to testing. A commercial mouse diet (pellets) and water (in glass water bottles equipped with stainless steel sipper tubes) were supplied *ad libitum*.

Test—Each experimental animal was hand held by the base of the tail, removed from its cage, and placed quickly and gently onto the surface of an individual glueboard. Mice held only by the tail base tend to splay their feet as they are lowered onto a surface; therefore, all four feet are readily brought into contact with the surface of a glueboard. Each mouse on a glueboard was immediately placed back into its cage after food and water were removed. The latter action was done to simulate field conditions in which a trapped animal would probably not be able to reach food or water resources. The control mice remained unhandled in their cages with food and water supplied *ad libitum*. A control glueboard was placed in an empty cage and weighed at the beginning and end of the 24-h test period.

Ten observations were made in each test: initial; 10-min intervals for 1 h; and at 3, 5, and 24 h. Observations were divided into five categories of "animal condition" and recorded for a particular time interval as described here:

1. Captured—given as a ratio of living to dead mice (living:dead).
2. Body part caught (includes both living and dead mice): (*a*) front feet—may include one foot or leg, but not the body; (*b*) hind feet—may include one foot or leg, but not the body; (*c*) body, trunk—includes any part of the body, but excludes tail alone; (*d*) head, face—includes any part of the head, face, and ears, but excludes vibrissae alone.
3. Responses (includes only living animals): (*a*) vocalizations—in the au-

dible range, spontaneous and without stimulation from observer; (*b*) struggling—spontaneously pulls, twists, stretches, and bites glue or glueboard.

4. Eye condition (includes only living animals): (*a*) normal—both eyes appear normal, or the same as in control mice; (*b*) pathological—for at least one eye, the eyelids and nictitating membranes (or both) become reddened and watery or covered with gelatinous exudate (clear or opaque).

5. Free—no body part caught on glueboard; given as a ratio of living to dead mice.

The multiple observation intervals, in addition to determining the glueboards' capture or holding ability *per se*, allowed us to monitor changes in animal condition that might be related to assessment of humaneness. Vocalizations and struggling (escape reactions) can be used as clinical evidence of pain or distress [7]. At the end of the 24-h test period, the surviving mice were euthanized.

Choice Procedure

Acclimatization—The experimental and reference animals (24 per group; sexes equal) were held group caged (sexes separate) in the laboratory for 24 h. The reference animals were then mixed (12 in each group; sexes equal) for the remainder of the study. The experimental mice were released together in the colony room two days prior to initiation of the pretest. The animals were not fasted or unduly stressed prior to the study. A commercial mouse diet (meal) and water were provided *ad libitum* throughout the choice test procedure.

Pretest—For four days preceding the test period, daily food consumption and activity counts for each tunnel were recorded at 24-h intervals. The main purpose of activity monitoring was to determine which two tunnels received the most activity, because these would become placement locations for glueboards during the test period.

Test—For testing, one glueboard was placed at each of the two tunnels (inside entrance) with the greatest activity counts recorded during the pretest. The boards were placed so as to just touch the lip of a tunnel entrance but were in no way fixed or held in place. The same two tunnels were used throughout the four test days, with a fresh glueboard placed each day at 3 p.m. after Actimeter readings were taken, food consumption recorded, and captive mice removed and examined. Also at this time, after 24 h of glueboard exposure, two animal conditions (captured and body part caught) were recorded as in the no-choice test; the living captures were then euthanized.

Glueboards were checked for captures at 2-h intervals between 9 a.m. and 3 p.m. each day; notations were made of captures, deaths, and evidence of escape from a board. A control glueboard was placed between the outer and inner walls (inaccessible to mice) of the choice-apparatus and checked for weight change at the end of each day.

Posttest—Observations (activity counts and food consumption) were continued for surviving, uncaptured mice for four days following the test period.

Results

The various types of data provided by this study are for exemplary purposes in designing test procedures. The different aspects that might be incorporated into a standard test protocol would depend on one's particular objectives (that is, capture success, holding power, or humaneness). Replicates of the tests should be carried out before firm conclusions are drawn regarding particular glueboard products.

Glueboard Characteristics

Control glueboards from both tests did not change weight, nor did their surface become obviously less sticky after 24 h of exposure. The adhesive material on Stick-Em boards had no perceptible odor whereas that on Trap-Stik boards had a strong petroleumlike odor which readily filled the testing rooms.

Both adhesives studied were sticky, but the Stick-Em material appeared to be more viscous and elastic than Trap-Stik. The latter material was more gelatinlike than the other and the surface could be touched lightly without sticking; the surface color of this adhesive was darker (perhaps oxidized) than the glue underneath. In some cases, a struggling mouse pulled up patches of Trap-Stik adhesive, thereby exposing areas (1 to 2 cm^2) of the base with only a very thin coating of adhesive and rendering such spots ineffective for capturing mice. The adhesive did not readily flow back over these exposed areas on Trap-Stik boards. With Stick-Em glueboards, the glue readily flowed into any area where it had been dislodged or otherwise disturbed. The physical and chemical characteristics of glueboards exposed to a range of temperature and humidity conditions likely to be experienced in the field are a subject deserving separate investigation. The establishment of criteria for viscosity, odor, color, and other perceptural qualities which might affect biological efficacy would be useful in the research and development of glueboard products.

No Choice

Data for no-choice tests are summarized in Tables 1 and 2. Once all four feet of a mouse were placed on a glueboard, the animal remained captured throughout the 24-h test period with both types of glueboard. Within 3 to 5 h after capture, most of the animals showed heavy defecation and urination (see Fig. 2); by 24 h, the wastes were often smeared over much of a mouse's body. These accumulated body wastes apparently did not reduce the glue's effectiveness in holding mice already captured.

TABLE 1—*Summary of the results for the no-choice test with albino laboratory mice (ten males; ten females) on Stick-Em glueboards.*

Animal Condition[a]	Number of Mice Exhibiting Condition at Observation Interval									
	0 min	10 min	20 min	30 min	40 min	50 min	1 h	3 h	5 h	24 h
Captured (living:dead)	20:0	20:0	20:0	20:0	20:0	20:0	20:0	19:1	19:0	17:2
Body part caught (living and dead)										
Front feet	20/20	18/20	20/20	20/20	20/20	20/20	20/20	20/20	19/19	18/19
Hind feet	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20	19/19	19/19
Body, trunk	2/20	10/20	18/20	19/20	20/20	20/20	20/20	20/20	19/19	19/19
Head, face	1/20	4/20	5/20	7/20	8/20	9/20	9/20	10/20	9/19	11/19
Responses (living only)										
Vocalizations	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/19	1/19	0/17
Struggling	8/20	5/20	4/20	3/20	5/20	5/20	8/20	0/19	1/19	4/17
Eye condition (living only)										
Normal	20/20	20/20	20/20	20/20	20/20	18/20	20/20	16/19	15/19	3/17
Pathological	0/20	0/20	0/20	0/20	0/20	2/20	0/20	3/19	4/19	14/17
Free (living:dead)	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0

[a]See text for definitions.

TABLE 2—*Summary of the results for the no-choice test with albino laboratory mice (ten males; ten females) on Trap-Stik glueboards.*

Animal Condition[a]	Number of Mice Exhibiting Condition at Observation Interval									
	0 min	10 min	20 min	30 min	40 min	50 min	1 h	3 h	5 h	24 h
Captured (living:dead)	20:0	20:0	20:0	20:0	20:0	20:0	20:0	19:1	19:0	18:1
Body part caught (living and dead)										
Front feet	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20	19/19	19/19
Hind feet	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20	19/19	19/19
Body, trunk	1/20	8/20	11/20	14/20	14/20	15/20	16/20	16/20	17/19	19/19
Head, face	0/20	0/20	0/20	0/20	0/20	0/20	0/20	2/20	2/19	6/19
Responses (living only)										
Vocalizations	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/19	0/19	0/18
Struggling	20/20	20/20	20/20	20/20	16/20	11/20	10/20	12/19	12/19	9/18
Eye condition (living only)										
Normal	20/20	20/20	20/20	20/20	17/20	16/20	12/20	10/19	8/19	3/18
Pathological	0/20	0/20	0/20	0/20	3/20	4/20	8/20	9/19	11/19	15/18
Free (living:dead)	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0

[a]See text for definitions.

Only the front feet of mice were observed to be freed, and only for a few minutes. More than half of all the mice had their bodies entangled in the adhesive after 20 min, and involvement of all bodies was noted after 24 h regardless of the product. Stick-Em boards had a greater proportion of the animals' heads entangled in the glue after 24 h than did the Trap-Stik boards. Some mice were able to move themselves about by use of a freed front leg or, when captured with their head over the edge of a board (see Fig. 2), by grabbing the floor with their incisors.

Vocalizations in the audible range were not significant in this study. Occasional vocalizations were heard, but they were neither loud nor persistent. Most animals struggled vigorously when initially placed on a glueboard. In an apparent effort to free themselves, animals stretched, twisted, and pulled on body parts caught in the glue; some mice bit and chewed on strands of adhesive or on their hair imbedded in the adhesive. This action often resulted in additional body parts becoming imbedded in the glue; freed front feet sometimes became recaught against the body or on the board. As a result of struggling, patches of hair were sometimes pulled out of the body or limbs, exposing bare, raw patches of skin; legs were sometimes broken by vigorous struggling.

Struggling was intermittent for any one animal and was observed throughout the 24-h test; however, by the end of the test half or fewer of the surviving mice responded vigorously to a puff of air blown into the face after 24 h, whereas virtually all newly captured mice exhibited a high-level response to this stimulus. The air was from a small bulb syringe, and it was difficult for the observer to be consistent in pressure applied to the bulbs.

The normal eye condition lasted for about 1 h in most animals. Pathological conditions developed most rapidly with Trap-Stik glueboards, but after 24 h the rates were very similar for both boards. We noted that the rate for severe eye pathology was highest with Trap-Stik after 24 h. Excessive watering occurred so that a wet, dark ring developed around the eye's orbit (see Fig. 2, upper mouse). This condition sometimes progressed further so that the eye and eyelids became covered with a thick, gelatinous exudate which, in its severest state, was milky white and could not be removed by blinking the eye.

The earliest deaths occurred after 3 h of capture on each type of board; a few additional deaths were found after 24 h (Tables 1 and 2), with no apparent differences between the two boards. Note that some animals, because of weakness (and in some cases due to struggling), fell face-first into the glue and apparently suffocated because of glue occluding the nostrils.

Choice

Table 3 summarizes the basic data for the two choice tests. Actimeters (hence, tunnels) 1 and 3 had the highest counts during the pretest period of both tests, and it was at these tunnels where the glueboards were placed for the test period. The proportion of activity was the same for both board de-

TABLE 3—*Summary of Actimeter counts and food consumption for choice tests with albino laboratory mice (twelve males; twelve females each) exposed to glueboards.*

Study Period	Mouse Days[a]		Actimeter Counts for Tunnels—4 Days						Food Consumption, g	
			1	3	2	4	Total	Average/ Mouse	Total	Average/ Mouse
			PART A—STICK-EM							
Pretest	96.0	total	9595	6984	4523	6550	27652	...	337.2	...
		average	2398.8	1746.0	1130.8	1637.5	...	288.0	...	3.5
		percent of total	60		40					
Test	64.5	total	5856	3376	3130	4333	16695	...	233.6	...
		average	1464.0	844.0	782.5	1083.2	...	258.8	...	3.6
		percent of total	55		45					
Posttest	36.0	total	4830	3254	3161	2978	14223	...	154.8	...
		average	1207.5	813.5	790.2	744.5	...	395.1	...	4.3
		percent of total	57		43					
			PART B—TRAP-STIK							
Pretest	96.0	total	10027	7467	4912	1944	28952	...	381.0	...
		average	2506.8	1866.8	1705.8	1158.8	...	301.6	...	4.0
		percent of total	60		40					
Test	84.0	total	7009	532	7292	3416	18249	...	382.2	...
		average	1752.2	133	1823	845	...	217.2	...	4.5
		percent of total	41		59					
Posttest	80.0	total	7076	5659	6830	3801	23366	...	403.3	...
		average	1769	1414.8	1707.5	950.2	...	292.1	...	5.0
		percent of total	54		46					

[a]Total number of active mice during study period; captured mice are considered to have been active for half of each night.

signs during pretesting (60%). Although the mice in both tests reduced their preference for Tunnels 1 and 3 during the test period, the reduction for Trap-Stik was dramatic, with a greater proportion of the activity (59%) shifting to tunnels without glueboards. During posttesting (after the glueboards were removed) activity at Tunnels 1 and 3 recovered somewhat in both tests, but to a lesser degree with the Trap-Stik test.

Table 4 shows that, with both glueboard types, a greater proportion of males was captured than females. However, Stick-Em boards captured a much greater proportion (0.62) of the mice than did Trap-Stik boards (0.17). An adjusted chi-square test with Yates' correction for continuity [9] indicated that the difference in the proportion of mice captured on the two boards was highly significant (χ^2 value = 8.71; $P < 0.005$). All the mice were captured overnight; among the captures, there were no obvious preferences for glueboard location.

In the morning, it was common to find glueboards moved completely away from their original placement (see Fig. 2). In one case, with Stick-Em, a mouse caught by only its hind feet early in the evening was able to pull the glueboard about the floor with its front feet and by morning had gotten its front feet caught on the second glueboard. Glueboards found moved and without captures could indicate that some animals escaped. With Stick-Em, one board was found overturned on the morning of Day 2, the surface of the board was disturbed and soiled with a few fecal pellets. A mouse may have been caught and then, while freeing itself, flipped the board over. Disturbed adhesive surfaces and bits of nest material or feces on the glue surface occurred with Trap-Stik boards on the morning of Day 3 (both boards) and Day 4 (one board); no mice were captured on either day, and the boards had been moved from their original placement.

Data regarding animal conditions observed after 24-h exposure (for each day of the four-day test period) are given in Table 5 though comparisons between board types are different since so few captures were achieved with Trap-Stik. Front feet, hind feet, and bodies tended to be caught in the glue after 24-h exposure regardless of glueboard type. The basic condition and appearance of the animals captured during the choice tests were comparable

TABLE 4—*Cumulative proportion of original mouse population captured each day (choice test).*

	Stick-Em			Trap-Stik		
Test Day	Males	Females	Total	Males	Females	Total
1	0.33	0.08	0.21	0.08	0.08	0.08
2	0.58	0.08	0.33	0.25	0.08	0.17
3	0.67	0.25	0.46	0.25	0.08	0.17
4	0.83	0.42	0.62	0.25	0.08	0.17

TABLE 5—*Summary of the animal condition results for albino laboratory mice (twelve males; twelve females per test) captured in the choice test.*

Animal Condition[a]	Number of Mice Exhibiting Condition After 24-h Exposure	
	Stick-Em	Trap-Stik
Captured (living:dead)	13:2	3:1
Body part caught (living and dead)		
Front feet	13/15	4/4
Hind feet	14/15	4/4
Body, trunk	14/15	4/4
Head, face	3/15	3/4

[a]See text for definitions.

to the descriptions given for the no-choice tests. No apparent differences were seen between the two boards regarding mortality rates of captured mice after a 24-h exposure (χ^2 value $= 1.80$; $P > 0.10$).

Figures for average food consumption per mouse (Table 3) approximately paralleled each other for the two glueboards, increasing slightly from pretest through posttest. These increases in food consumption may, in part, reflect the fact that more than half of the surviving females of each test were obviously pregnant (visual/palpation determination) at the end of the study.

Discussion

The two laboratory test designs provided, with reasonable speed, alternative and overlapping data to evaluate the competency of glueboards designed to capture mice. The no-choice test evaluates holding ability; that is, will a glueboard hold a mouse captive for at least 24 h once all four of its feet are brought into contact with a glueboard's adhesive? Products showing positive results from this screening procedure are then exposed to mice in the choice situation. This latter procedure evaluates the extent to which free-ranging mice will go onto a glueboard and become caught, an indication of how well a product might perform in a field situation. That is, these animals are free-ranging, social interactions occur, and they choose whether to approach their food supply via a route with or without a glueboard. The proportion of mice captured after a fixed time period, for example, four days of continuous glueboard exposure, can be used to compare products.

A simple measure of the holding ability and capture success at fixed intervals could be all that is necessary for an efficacy evaluation; however, our method allows for a combination of measurement strategies depending on the testing requirements. Of course, any efficacy testing of a product should include data for wild mice which may respond differently from laboratory animals.

It is important to know how long an animal might remain captured once it is caught on a glueboard; this is most practically assessed in the no-choice situation, in which the exact time of capture is known. The purpose of a test will determine the observation interval used. Since we did not know how long an animal might stay captured or how rapidly conditions would occur or change, we used multiple observations. Although a single observation after 24 h could be used, the unpredictable responses of mice to different products would limit the usefulness of the data. We suggest that no-choice observations be recorded at several intervals, such as at 0, 1, 5, and 24 h. Since a mouse on a glueboard might free itself and later become recaptured in the confinement of its cage, the frequent observations would allow such events to be detected. No escapes were observed in the no-choice tests, but in the choice tests there was evidence (for example, overturned boards, disturbed adhesive surfaces) that some mice probably freed themselves after being caught (by fewer than all four feet). From our work with Stick-Em and Trap-Stik, it appeared that when at least two limbs were caught, other body parts also became entangled as the animal struggled to free itself. Since the tackiness, viscosity, and depth of adhesive vary with different products, it would be useful to investigate how much of a mouse (one foot, and so on) must contact the adhesive surface of a particular product in order for the animal to be permanently caught.

A reason for using a 24-h test (or maximum exposure) was that a glueboard should hold a mouse at least that long for practical control purposes. It is also prudent to check glueboards frequently, for example, daily [*10*], since their effectiveness may be reduced by surface contamination (dust, soil, feces) and temperature extremes [*4*]. With the glueboards studied, surface contamination with urine and feces occurred (after a mouse was held captive for several hours) and apparently did not affect holding ability. However, such contamination would undoubtedly reduce the probability of additional captures if captives were left on the small glueboards much over 24 h. With 24 h, multiple captures were common in the choice test, but some boards were becoming excessively covered with body wastes. These data also suggest that the presence of a captive mouse does not deter others from being caught, and the authors (unpublished data) have observed multiple captures in field applications with mouse glueboards.

The test period for the choice study was restricted to four days since mice are reported [*2,11*] to become wary of traps laid out for many days. There was no significant development of trap shyness with the Stick-Em glueboard (with high capture success), and perhaps its exposure could have been extended without reducing its effectiveness. However, glueboards should probably be closely monitored, and it has been shown with other types of mousetraps [*2,10,11*] that frequent trapping with many traps for a few days is more effective than distributing traps sparsely over a wide area for weeks at a time. Using four days of pretest, test, and posttest made it possible to compare activity (in tunnels leading to the food and water supply) at the different time

periods and thereby detect an avoidance response to one of the products studied. The capture success with Trap-Stik was low, but it was the activity data which verified that mice had shifted their activity away from tunnels with glueboards. Thus, continuous monitoring of activity in the choice test provides valuable data. Monitoring equipment other than Actimeters could be employed but must not interfere with mouse behavior or with the functioning of glueboards.

To some degree, the observations of vocalizations, struggling, and eye condition were qualitative, but there were also practical considerations. The eye dysfunctions were probably indicative of stress (similar to that observed in immobilization experiments with rodents), varied somewhat with the products tested, and may also have been related to volatile products contained in the adhesive material. The extreme form, when eyes are coated with white gelatinous exudate, may be aesthetically disturbing to the public. Vocalizations of captive mice were noted for similar reasons, and it was also thought that such responses might repel other mice. In the laboratory mice studied, vocalizations (in the audible range) were uncommon and enough multiple captures occurred to indicate that vocalizations were probably not important. This factor might play a more significant role with wild mice and should be investigated.

It has been suggested [*12*] that the weight of glueboards prevent mice from moving them. In our studies with two commercial products, we found that $\simeq$23-g mice commonly moved $\simeq$30-g glueboards from their original placement by use of a free foot and their teeth (when the head was free), or both. Movement of glueboards by struggling mice could be prevented by tacking or taping a glueboard in place, but this would then allow more leverage for a mouse when pulling on nearby objects and could increase the chances of escape. Unless a board can be located on a flat smooth surface, it should probably not be fixed in place. The following are some additional considerations: a moving glueboard might create a disturbing noise for a homeowner; a board might be moved to a less effective location; and the public might become concerned if mice move themselves into view [*12*] or into areas where pets or children might become involved [*10,13*].

Mortality rates within 24 h were insignificant for laboratory mice; with additional time on the glueboards, more animals would undoubtedly die due to stress, suffocation, and starvation. Mortality of wild house mice captured on glueboards was not studied, and they may undergo greater stress and die faster than laboratory mice; it is indicated on packages of Stick-Em glueboards that mice "usually die within a few hours." If mortalities are not high within 24 h, the homeowner or pest control operator would need to deal with live captives. In addition, the extended lag period until death reflects on the humaneness factor for glueboard products.

The animal welfare issue regarding rodenticides has been raised in at least Canada and Great Britain and has received some attention in the United

States with regard to glueboards[2] [*2, 7, 13*]. Although there are no standards for humaneness of pest control devices, they should produce rapid unconsciousness without distress, though the lag time until death may be delayed [*7*]. It has been suggested that poisons which stress (conscious) animals for as much as 24 h are not humane. Half or more of the mice in the no-choice test still exhibited clinical evidence of pain or distress (such as escape reactions) after 24 h of capture. The appearance of the remaining animals, though often aesthetically disturbing, was not necessarily indicative of stress (for example, stress-induced analgesia might occur during extended periods of immobilization). More information is needed on this subject, particularly with wild house mice. Understandably, it is difficult to be humane with glueboards (as with some other traps [*1*]), which are designed to hold mice against their will until death.

Summary

The major conclusions and recommendations of this study are as follows:

1. A two-step procedure for laboratory evaluation of mouse glueboard efficacy was developed and successfully tested.
2. The holding ability of glueboards can be assessed with a 24-h no-choice test in which mice are placed with all four feet in contact with a glueboard surface.
3. Capture success can be evaluated by allowing free-ranging mice a choice of four access routes (two with glueboards) to food for a four-day period.
4. Continuous activity monitoring during the choice test is a valuable asset in detecting avoidance responses.
5. Mortality rates after 24 h of captivity, or of glueboard exposure, are easily measured in the procedures described and were not significant in this study.
6. The presence of a captive animal does not deter other mice from being captured on a glueboard.
7. Glueboards can be an effective supplementary tool for reducing mouse populations but vary in their control efficacy due to design characteristics, which include adhesive tackiness, viscosity, odor, and depth.
8. The use of glueboards has negative aesthetic considerations, and there are indications that these control devices may be inhumane. In the authors' opinion, glueboard application should be limited to problems for which other management strategies are inappropriate or have failed; and they should be monitored at 24-h intervals.
9. It is recommended that the industry develop glueboards constructed of

[2]D. E. Kaukeinen, ICI Americas, Inc., Biological Research Center, Goldsboro, N.C., personal communication, 1 Dec. 1980.

biodegradable materials (that is, no plastic base) to enhance disposal and not contribute to solid waste pollution.

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Donald J. Elias,[1] *Peter J. Savarie,*[1] *David J. Hayes,*[1] *and Michael W. Fall*[1]

A Simulated Burrow System for Laboratory Evaluation of Vertebrate Control Fumigants

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ABSTRACT: A simulated burrow system for laboratory testing of vertebrate pest control fumigants was constructed of readily available plastic pipe and other inexpensive materials. The device is versatile in that it can be easily modified to different configurations. In addition, the device permits controlled comparative evaluation of fumigant agents, test animals can be directly observed during exposure, access to them is immediate, and fumigant dispersal and concentrations can be monitored quickly and accurately.

KEY WORDS: vertebrate pest control, fumigants, rodents, simulated rodent burrow, pyrotechnic fumigant

Toxic gases have been used as fumigants for vertebrate pest control for many years. Methyl bromide, calcium cyanide, chloropicrin, aluminum phosphide, and carbon monoxide (primarily from internal combustion engines) are the more commonly used and well-known fumigants. Although numerous references to these materials can be found in the literature [*1–3*], there is a relative paucity of efficacy data available, especially under laboratory conditions. This fact is of special significance because strict regulations covering the manufacture, sale, and use of these and other pest control agents are in effect. Future development of such materials may require large amounts of laboratory and field data to meet regulatory agency registration requirements. Unfortunately, no specific testing standards or procedures have been established

[1]Wildlife biologist, pharmacologist, biology technician, and wildlife biologist, respectively, Denver Wildlife Research Center, Denver, Colo. 80225.

for laboratory evaluation of fumigants. Even the draft guidelines promulgated by the U.S. Environmental Protection Agency (EPA) are generally vague.

Most fumigant evaluations, as described in the literature, have been field trials. Although efficacy under field conditions is the ultimate goal of any control method, trials in the laboratory can, more easily and at considerably less cost and effort, provide information that is difficult or impossible to obtain in the field. To this end, we have designed and constructed burrow systems for laboratory evaluation of fumigants for predators [that is, coyotes (*Canis latrans*)] and rodents. The basic principles, techniques, and auxiliary equipment used are the same, so this discussion will be confined to the rodent system. We were primarily interested in developing a fumigant for control of the bandicoot rat (*Bandicota bengalensis*) in Bangladesh. This species causes considerable damage to agricultural crops such as wheat and rice in Bangladesh, Nepal, and other countries of South Asia. Hence, the bandicoot burrow served as the model for our system.

Methods

The physical dimensions and characteristics of the simulated rodent burrow (SRB) are based on average measurements obtained during the excavation of 20 *B. bengalensis* burrows in Bangladesh [*4*]. Several characteristics of the actual and simulated burrows are compared in Table 1.

The SRB is constructed primarily of 76.2-mm (inside diameter) plastic [polyvinyl chloride (PVC)] pipe and corresponding elbow, T, and other joints (Fig. 1). A 1.22-m section of copper pipe (76.2-mm inside diameter) is used at the ignition point and 3.79-L (1-gal) cans are used to simulate nest or storage chambers. The PVC pipe and corresponding elbow, T, or other joints slip together (or apart) easily. They are well matched and we experienced few incidents of fumigant leakage. Duct tape adequately sealed those few leaks that did occur. Appropriately sized openings were cut into the lids and bottoms of the cans

TABLE 1—*Comparison of characteristics of actual and simulated* Bandicota bengalensis *burrow systems.*

Characteristic	*B. bengalensis* Burrow ($\bar{X}$ values)	SRB
No. of entrances	6.4	6
No. of plugged entrances	3.9	0 to 6 (as desired)
Total length, m	40.2	40.2
Burrow diameter, mm	76.9	76.2
Depth, cm	16.7	...
Number of branches	27.6	28
$\bar{X}$ distance between branches, m	1.1	1.2
No. of dead ends	4.2	4
No. of storage chambers	2.5	4
No. of nest chambers	1.5	

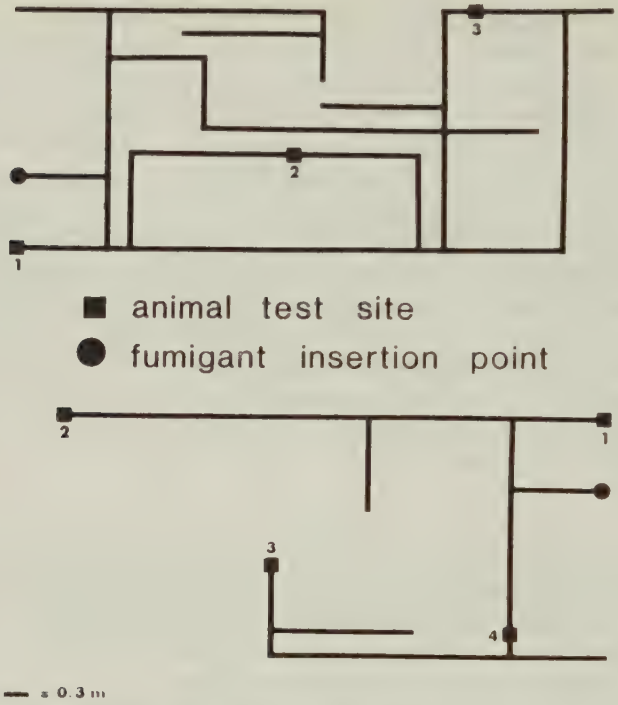

FIG. 1—*Diagrams illustrating the versatility of the SRB. The upper drawing is the configuration that corresponds to the bandicoot burrow with a total volume of 0.2 m^3 (7.11 ft^3). The bottom diagram is a modification that was used for a series of tests and contains 0.09 m^3 (3.32 ft^3).*

used as nesting or storage chambers. The plastic pipe was inserted about 1 cm into the opening and fixed in place with construction adhesive (such as Liquid Nails[2]). Access to the chambers was accomplished by removal of the lids with a paint can opener. The copper pipe was necessary because temperatures generated by pyrotechnic fumigants would melt the PVC pipe. The copper pipe was affixed to a PVC joint with duct tape. The can which served as the fumigant insertion point was soldered to the copper pipe. Clear sections of plastic tubing are used at various points to permit observation of test animals, and clear plastic windows incorporated into the chambers likewise facilitate observation. Test animals can be held and observed at eleven points in the system, and simple modifications can be made to expand this capacity if needed. The burrow entrances can be open or closed during testing, and animals can be restrained at the fixed observation points or allowed free movement within the burrow. Measurements of oxygen or toxic gases can be made anywhere along the system by using Draeger gas measuring tubes (National Draeger, Inc.[2]) in-

[2]Reference to commercial products does not imply government endorsement.

serted into self-sealing ports. These ports are constructed by drilling a 1-cm opening at the desired location and covering it with surgical latex rubber. A pinhole in the rubber will expand to admit the Draeger tube and subsequently close sufficiently to prevent escape of the fumigant. The total volume of the system is 0.2 m^3 (7.11 ft^3).

Results and Discussion

There are few references to artificial burrow systems or laboratory evaluation of fumigants in the literature [*3, 5–7*]. In our opinion, such evaluations are a necessary and logical step in the development of a fumigant for vertebrate pest control. Consider, for example, the figures in Table 2. We were evaluating different formulations (that is, percentages) of the active ingredients in a pyrotechnic fumigant cartridge. All other conditions during the trials were the same. The difference in efficacy is obvious and consistent, and these tests, along with others, helped us find the best formulation for eventual field testing.

The simulated burrow system we devised has a number of advantages. For example, it will provide initial efficacy data; permit comparative evaluations, under controlled conditions, of different formulations or materials; permit direct observations of animals exposed to the agent; allow precise measurement of fumigant concentration at different points within the system over time; make replication of tests easy; permit quick and easy modification of the configuration and size of the system; allow immediate access to the test animals for postmortem examination or use in secondary hazard evaluations; and permit use indoors where certain variables, such as temperature, can be controlled. It is simple to clean, move, and store; it is not easily destroyed or damaged either by the test animals or an accident; and it is inexpensive.

Our device shares a disadvantage common to all laboratory animal testing apparatus: it is an artificial environment. The test animals are probably under greater stress, and the dispersal and fate of the fumigants is not as it would be in a natural burrow.

TABLE 2—*Comparative efficacy of two fumigant cartridge formulations.*[a]

Formulation	Site 1	Site 2	Site 3[b]
65% $NaNO_3$, 35% C[c]	24 (10 to 48)	63 (25 to 105)	115 (80 to 190)
50% $NaNO_3$, 50% C[d]	31 (20 to 45)	114 (50 to 270)	>263 (170 to 410)[e]

[a]The average time to death (range) in minutes is given for albino rats at three locations in the SRB *Bandicota* configuration.

[b]Sites 1, 2, and 3 are 3.4, 6.1, and 10.1 m (11, 20, and 33 ft), respectively, from the point of introduction of the fumigant.

[c]Based on six trials.

[d]Based on eleven trials.

[e]The average does not include three animals that died overnight.

Conclusion

Despite the artificiality of this and a similar system used for predators, the results of tests conducted in them provided information needed for development of an effective pyrotechnic fumigant cartridge [*8*]. The resultant device has been approved by the EPA for use against denning coyotes (EPA Reg. No. 6704-84), and efforts will be undertaken to obtain similar registration for burrowing rodents.

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Parvin Sultana,[1] *Joe E. Brooks,*[2] *and Richard M. Poché*[2]

Methods for Assessing Rat Damage to Growing Wheat in Bangladesh, with Examples of Applications

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ABSTRACT: Methods for assessing damage by the lesser bandicoot rat, *Bandicota bengalensis*, to growing wheat crops are described and compared with other methods. Damage was related to yield, density of wheat stems, agricultural practices, and the foraging range of the rodent species. Applications of the damage assessment are given for estimating the annual losses to growing wheat over several large wheat districts in Bangladesh, and uses of the assessment in evaluating the efficacy of rodenticides in field trials and farmer use of rodenticide treatments are shown.

KEY WORDS: damage assessments, wheat, Bangladesh, crop loss estimation, rodenticides, evaluation methods, efficacy, *Bandicota bengalensis*, zinc phosphide, difenacoum, brodifacoum, calcium cyanide, vertebrate pest control

The methodologies for rodent damage assessments in wheat are still in the developmental stages. Bindra and Sagar [*1*] did some work on wheat losses in India, and Fulk et al [*2*] reported on similar studies in Pakistan. Studies of rat damage to growing wheat crops in Bangladesh were carried out by Haque et al [*3*], Poché et al [*4*], and Posamentier and Alam [*5*], and methods of rodent control were developed by Poché et al [*6*]. Throughout most of the wheat growing areas of South Asia the primary rodent pest is the lesser bandicoot rat, *Bandicota bengalensis*. This species digs elaborate burrow systems and caches large amounts of grain, both rice and wheat.

[1]Senior scientific officer, Vertebrate Pest Section, Bangladesh Agricultural Research Institute, Dacca, Bangladesh.

[2]Wildlife biologist and former wildlife biologist, respectively, U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Denver, Colo. 80225.

The purpose of the present paper is to outline the methods used in Bangladesh for assessing rat damage to wheat and to show how these methods were used to quantify the extent of rodent damage, to determine the effects of stem cutting on yield, and to demonstrate other applications of damage assessment methods.

Methods

Wheat fields were selected for sampling by random sampling procedures. For determining rat damage to wheat in major wheat-producing districts in Bangladesh, four districts were first chosen [*4*]. Eight thanas (a smaller administrative jurisdiction within a district), two from each district, were selected randomly from district maps, and villages within each thana were numbered. Lots were drawn and four villages chosen per thana. Eight wheat fields were then selected from each village by using the cardinal compass directions and sampling at 100 and 200 m from the village center along each transect line. The field lying nearest the transect line was selected.

Within wheat fields, ten 50- by 100-cm quadrats were examined for rat damage [*4*]. Wooden frames were used to delineate the quadrats. Two rows of five quadrats were laid out in accordance with the length and width of the field and then sampled. Total stems and the number of rat-damaged stems were counted within each quadrat. Bandicoot rat damage to wheat stems was of two types: the stem was bent over and cut at the base of the panicle or the stem was cut about 4 to 15 cm above the ground, the plant felled, and the panicle then removed and cached in the burrow [*4*]. Lesser bandicoot rat foraging was most intense near the burrow openings; the heaviest feeding was within 2.5 m of the burrow system center and decreased sharply beyond 6 m [*4*]. Rat damage is, accordingly, clumped within a field.

The effect of rat stem cutting on wheat yield was evaluated in one thana by preselecting 20 fields with varying degrees of rat damage [*4*]. Field dimensions were measured in metres and the diagonal field length was determined. Along one diagonal, five evenly spaced square-metre quadrats were examined and the number of cut and uncut stems was counted. All panicles on each quadrat were harvested, deposited in plastic bags, and labeled. They were threshed by hand, sun dried for 8 h and weighed to the nearest gram. The observed yield, Y_0, and potential yield, Y_p, were compared for each field. The potential yield was calculated as

$$Y_p = Y_0 \frac{N}{n}$$

where Y_0 was the weight of grains harvested, n was the number of undamaged panicles, and N was the total number of damaged and undamaged panicles [*7*].

We assessed rat damage reduction to farmers' fields following farmer use of

prepackaged baits in Gazaria Thana by first selecting 12 villages at random from the 48 total villages in the thana [*8*]. At each village, transects were laid out in three directions at 0, 120, and 240°. Along each transect line, the wheat fields closest to a 100-, 200-, and 300-m distance from the village were selected, giving a total of nine fields per village.

Within each field, the nearest corner was chosen and a diagonal line to the opposite corner was followed. Five steps along this diagonal line were paced off and a 50- by 100-cm area was sampled for both damaged and undamaged wheat stems. This was repeated until five quadrats had been sampled.

Results

Countrywide Wheat Damage, 1979

Rat damage to wheat was assessed in four districts in Bangladesh in 1979 [*4*]. Only 0.5% of the stems were cut by rats before the booting stage. The average percentage of cut stems for all fields remained under 1% until the crops attained an age of more than 60 days. Between 60 and 120 days after sowing, rat damage increased from 2 to 12% (Fig. 1). Dacca district had the highest dam-

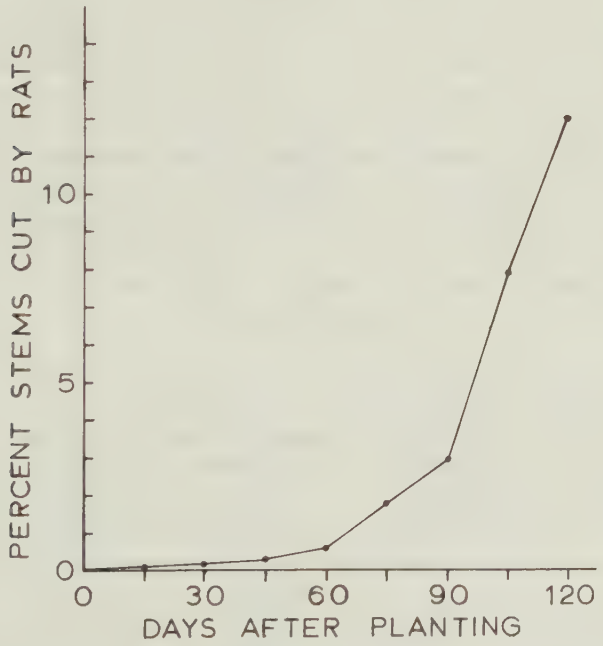

FIG. 1—*The accumulation of rat damage in fields during the 1979 wheat-growing season in Bangladesh (taken from Fig. 2 in Ref 4).*

age (14%) and Jessore the lowest (7%). The overall damage in mature wheat for the four districts was 12.1% (Table 1).

Relationship of Damage and Yield

A study of damage and yield was carried out in Gazaria Thana [*4*]. A summary of the data is given in Table 2. A total of 567 (17%) of the wheat stems damaged by rats had only the panicles removed, whereas 2725 (83%) were cut within 15 cm of the ground surface. Panicles fit more easily into burrow storage chambers if the excess stem is removed. As shown in Fig. 2, there was a strong relationship ($r = 0.978$) between rat-damaged stems and yield loss, expressed by the regression formula, $Y = 13.12x - 17.38$.

Rodenticide Evaluations

Four rodenticides were evaluated [*6*] as rodent control agents in wheat fields: calcium cyanide (a fumigant), difenacoum bait (0.005%), brodifacoum bait (0.005%), and zinc phosphide bait (2%). The baits and fumigant were applied in fields beginning at the booting stage of crop maturity, and applications were made weekly thereafter.

Five study plots were selected, each containing five to eight contiguous wheat fields and varying in total area from 0.68 to 0.89 ha (Table 3). The wheat fields in each plot were sampled for rat damage in the mature crop, 12 to 14 weeks after sowing [*6*], using the ten 50- by 100-cm quadrats per field, as previously described. The observed results of the efficacy of each rodenticide are given in Table 3. The control plot suffered ten times more damage than the fields treated with difenacoum. The brodifacoum-treated plot received the greatest amount of damage. These fields, however, were located adjacent to a potato field which had numerous active lesser bandicoot rat burrow systems. The potatoes were harvested two weeks before the wheat crop, at which time many of the rats moved into the brodifacoum plot.

TABLE 1—*Rodent damage to wheat crops in Bangladesh, 1979.*[a]

District	Fields Sampled	Total Stems Counted	Cut Stems	Percent Damage
Dacca	48	58 226	8 152	14.0
Comilla	21	28 896	3 756	13.0
Pabna	24	24 581	3 073	12.5
Jessore	26	28 743	2 012	7.0
Total/average	119	140 446	16 993	12.1

[a]Taken from Table 1 in Ref *4*.

TABLE 2—*Relationship between the level of rat damage and yield in 20 fields of Gazaria Thana.*[a]

	Total Stems	Cut Stems	Percent Damage	Observed Yield, Y_0	Potential Yield, Y_p	Loss
	554	0	0.0	512	512	0
	716	2	0.3	760	762	2
	971	13	1.3	850	862	12
	1399	35	2.5	956	980	24
	776	23	3.0	776	800	24
	1171	60	5.1	753	794	41
	1003	66	6.6	789	844	55
	987	87	8.8	936	1026	90
	1537	138	9.0	1028	1129	101
	1294	117	9.0	1100	1209	109
	1560	144	9.2	1007	1109	102
	1225	113	9.2	1073	1182	109
	1455	176	12.1	1028	1170	142
	1028	132	12.8	701	804	103
	1255	170	13.5	896	1036	140
	1835	261	14.2	1119	1305	186
	1781	382	21.4	831	1058	227
	1828	464	25.4	811	1087	276
	1755	463	26.4	1075	1460	385
	1489	446	30.0	958	1368	410
Average	1281.0	164.6	12.8	898.0	1024.9	126.9

[a]Data from 5-m^2 quadrats each field. Yield and loss are expressed as grams per 5 m^2. Modified from Fig. 5 and Table 2 in Ref *4*.

A linear regression analysis of the treated plot data [*6*], examining stem density, x, and percentage of rat damage, y, gave the equation $y = 0.0122x - 3.024$ ($r = 0.92$, $P < 0.05$). This indicated there was little observed difference between the control materials tested and the rat damage correlated with stem density.

Farmer Use of Prepackaged Rodenticide Baits

Seven hundred packets, each containing 100 g of 2% zinc phosphide bait cakes, were made up at the Vertebrate Pest Laboratory, Joydebpur, Bangladesh, and were sold at cost in the central market in Gazaria Thana to wheat farmers during the 1981 wheat season. Just before harvest, wheat fields were assessed at random for rat damage. After damage assessment, it was determined whether the farmer had used the prepackaged bait, had used other rodenticide bait, or had conducted no control [*8*].

A summary of the results of the survey of 108 wheat fields is given in Table 4. Twenty farmers (18.5%) had used the prepackaged zinc phosphide bait in their fields. Their damage level was only 28% of that of the farmers using other rodenticide baits or doing nothing [*8*]. A reduction in damage was achieved

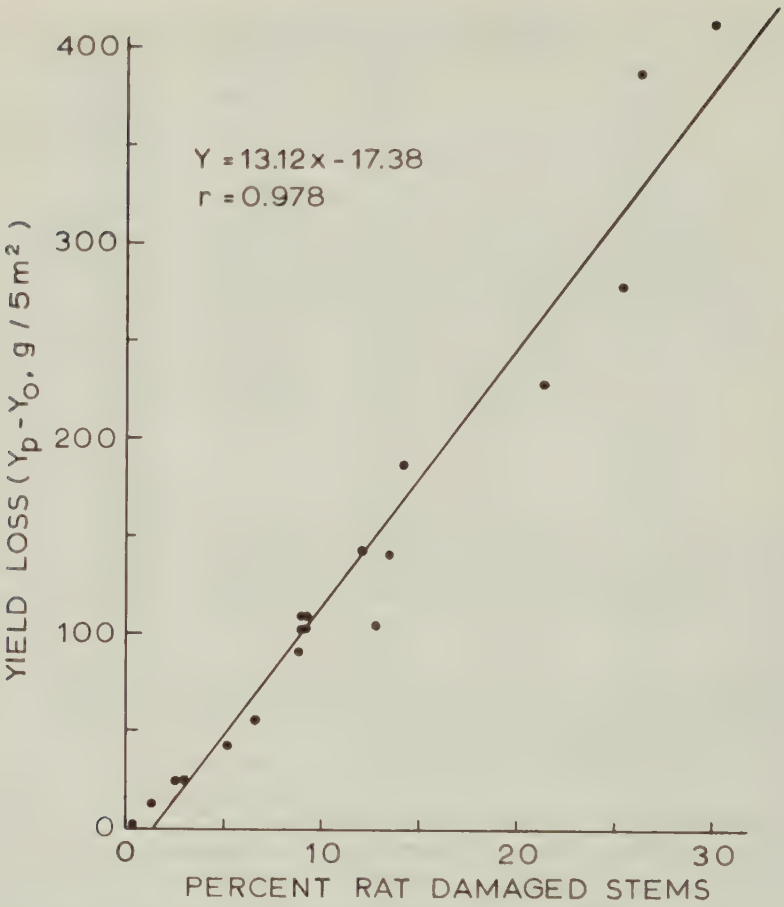

FIG. 2—*Regression line illustrating the relationship between rat damage to wheat stems and yield loss (modified from Fig. 4 in Ref 4).*

TABLE 3—*Summary of data for rodenticide field trials in wheat fields.*[a]

	Calcium Cyanide	Difenacoum	Zinc Phosphide	Brodifacoum	Control
Number of fields	8	8	6	5	6
Total area, ha	0.89	0.68	0.75	0.69	0.72
Number of quad-rats sampled	46	32	46	24	50
Uncut stems	6561	4436	7579	4055	8148
Cut stems	38	13	63	53	262
Total stems	6599	4449	7642	4108	8410
Percentage rat-damaged stems	0.58	0.29	0.82	1.29	3.10

[a]Data are from Ref *6*.

TABLE 4—*Rat damage observed in wheat fields treated by farmers in Gazaria Thana with prepackaged zinc phosphide baits, other rodenticide baits, or no poison.*[a]

Treatment	Number of Farms	Cut Stems	Total Stems Counted	Percent Damage
Zinc phosphide	20	334	12 752	2.6
Other rodenticide	8	432	4 820	9.0
No poison	80	4480	48 523	9.2

[a]Data are from Ref *8*.

with very little supervision of the farmer. Our only contact was to give simple instructions at the time of bait sale.

Discussion

Fulk et al [*2*] traveled a predetermined auto route in surveys of rodent damage to wheat in Pakistan. They stopped at intervals of 10 to 20 km, and at each stop three to five field workers each walked a straight line (transect) through the crop. As they walked, they placed a 1-m^2 frame at 5- to 10-m intervals at 25 locations. At each quadrat they examined the wheat stems for rodent damage and estimated the percentage of rodent damage in six damage classes: 0 = no damage, 2.5 = 0.1 to 5% cut stems, 15.0 = 5 to 25%, 37.5 = 25 to 50%, 62.5 = 50 to 75%, and 87.5 = 75 to 100% cut stems.

These estimates were standardized by the field workers during a one-day training period by counting the actual number of cut and uncut stems in five quadrats per transect. After several hours the workers could estimate with accuracy. From their subsequent field estimates, they derived the percentage damage for all fields examined in 1979 ($n = 294$) at 2.0%. They used the damage frequency (x = number of quadrats with damage) to convert estimates of the percentage damage, y, for data collected in 1978 by the regression formula $y = 0.14x - 0.83$. This gave a damage estimate of 2.8% rat damage in 1978.

The field estimates and the grouping of the fields by damage classes are not as accurate as counting the actual cut and uncut stems, and they allow less precision in estimating the reliability of the data. The irregular spacing of the quadrats (5 to 10 m) along the transect line can lead to unconscious bias on the part of the field worker to select areas with obvious damage, leading to a higher damage estimate.

Posamentier and Alam [*5*] used ten quadrats per field spaced equidistant on a transect along one of the field diagonals. The quadrat size was 0.093 m^2, giving a total of 0.929 m^2 sampled per field. Damage assessments carried out in this manner in the 1980 wheat-growing season in 433 fields in 20 thanas in Bangladesh showed only 2.5% rat damage. This same year in Gazaria Thana we observed only 3.2% rat damage in an area where the year before damage had averaged 12.8% and that in the year following was 9.2%. This indicates good

agreement between the data. We believe, however, that the use of the 50- by 100-cm sampling quadrat (which is 5.4 times larger than the 0.093-m^2 quadrat) gives greater accuracy. The time and expense involved in selecting and then visiting the fields are considerable, so the little extra time devoted to counting stems in a larger quadrat is well justified, just as making stem counts is superior to simply making percentage estimates, as the workers in Pakistan did. The issue of the size of the quadrat is, however, open to testing, and an optimum size should be determined.

The importance of these damage assessment methods lies (1) in the random selection of the fields to be surveyed, whether by picking villages at random or by driving a preselected route and stopping at regular intervals, (2) in setting the quadrats at regular intervals based on the length of a field or its diagonal, (3) in counting all the stems, cut and uncut, and (4) in timing the survey to coincide with the last week or two before harvest. This results in data that are relatively free from bias and can be statistically analyzed in several ways.

Acknowledgments

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Kathleen A. Fagerstone[1]

An Evaluation of Visual Counts for Censusing Ground Squirrels

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ABSTRACT: Visual counts frequently are used to census ground squirrels before and after a control program. However, the precision of visual counts is not known. During 1979 and 1980 I compared visual counts of Richardson's ground squirrels *(Spermophilus richardsonii elegans)* with mark-recapture population estimates on a study area near Kremmling, Colo. There was no significant correlation between the population estimate generated over a five-day trapping period and the highest visual count on a particular day within that period ($R = 0.42$, $P = 0.06$). However, there was a significant correlation between the population estimate and the highest ground squirrel count during the five-day period ($R = 0.65$, $P = 0.009$). Therefore, if certain guidelines are followed, visual counts may be reliable indices of ground squirrel populations.

KEY WORDS: mark-recapture, population estimates, Richardson's ground squirrels (*Spermophilus richardsonii elegans*), vertebrate pest control, visual counts

Ground squirrel population numbers have been estimated using a variety of methods [*1*], including visual counts [*2,3*]. Visual counts frequently are used in conjunction with control programs, in which pretreatment and posttreatment counts are compared to determine the effectiveness of rodenticide applications to ground squirrel populations [*4–6*]. Ground squirrels are well suited for visual counts because of their large size, their diurnal activity patterns, and their tendency to live in social colonies. The objective of this study was to compare the precision of visual counts of Richardson's ground squirrels (*Spermophilus richardsonii elegans*) with mark-recapture population estimates.

Direct visual counts are fast, inexpensive, and not labor intensive. However, visual counts are difficult to replicate because different observers counting at

[1]Research biologist, U.S. Fish and Wildlife Service, Denver Wildlife Research Center, Denver, Colo. 80225.

the same place and time will count different numbers of animals (Fagerstone, unpublished data). Also, the precision of visual counts may be low because of the inability of an observer to distinguish between resident and migrant squirrels and because of natural variation in the number of squirrels above ground.

Procedure

The study area was in northcentral Colorado, in Grand County, 8 km north of Kremmling. Throughout this area, Richardson's ground squirrels have established a nearly continuous series of colonies. I chose to study a 5-ha colony located on a sparsely vegetated pasture dominated by brome (*Bromus* spp.) and pigweed (*Amaranthus graecizans*) and surrounded by sagebrush (*Artemisia tridentata*) rangeland. The elevation at the colony site is 2350 m.

Mark-Recapture

I censused ground squirrel populations by the mark-recapture method at two-week intervals from May to September 1979 and from April to September 1980 [*7*]. I established a 90- by 90-m grid on the 5-ha study colony, using 100 National live traps (15 cm by 15 cm by 41 cm) located at 10-m intervals. Before each trapping period, the traps were wired open and baited with sorghum grain for a two-day familiarization period. During the subsequent five-day trapping period, the traps were baited with sorghum and checked in the morning and evening; they were closed during midday to avoid ground squirrel mortality caused by heat stress.

The trapped ground squirrels were aged (juvenile, yearling, or adult), sexed, weighed, and ear tagged with Monel No. 1 fingerling fish tags. Each squirrel was then painted with Nyanzol D dye [*8*] in a unique pattern of spots. In addition, colored neck collars identified each animal by sex and age [*7*]. The ground squirrels were released at the point of capture.

I computed population estimates for each of the trapping periods using the computer program CAPTURE [*9*]. Otis et al [*10*] have provided a detailed reference on the theory behind program CAPTURE.

Visual Counts

Ground squirrels were counted from a 5-m-high wooden tower erected in the center of the trapping grid. The ground squirrels were counted at hourly intervals from 6 a.m. to 8 p.m. for two or three days each week during 1979 and 1980. For each count, one systematic 360° scan of the grid was made with binoculars and a $\times 15$ to $\times 45$ variable-power spotting scope. Most counts averaged about 20 min, depending on the number of ground squirrels above ground. Each ground squirrel observed was individually identified by its collar color and dye marks. An individual ground squirrel was counted only once.

Statistics

Two correlation coefficients were computed (1) between the highest count of individual ground squirrels for any scan during one day and the population estimate generated by program CAPTURE for the corresponding five-day period, and (2) between the highest single count of individual ground squirrels over a five-day period and the population estimate for that period.

Results

On 49 days during 1979 and 1980 I made visual counts immediately before, during, or immediately after a five-day trapping period (Table 1). The correlation between the highest count on a day and the population estimate for the corresponding period was low ($R = 0.42$, $P = 0.06$). Therefore, the highest number of ground squirrels counted above ground during one day does not appear to be a good determinant of the size of a ground squirrel population. On any given day too much variation is present in both the number trapped and the number active above ground to provide meaningful information on population numbers.

The correlation was higher ($R = 0.65$) between the population estimate for a five-day trapping period and the highest number of animals counted visually in a single sweep during that period (Table 2); this was statistically significant ($P = 0.009$). Visual counts conducted for two or three consecutive days may, therefore, provide a useful index to ground squirrel population numbers.

Discussion

Mark-recapture is a fairly reliable method for estimating the abundance of Richardson's ground squirrels because these squirrels are readily trapped and have relatively small home ranges. The estimates of abundance using mark-recapture and visual counts were independent of one another and produced comparable results in this study. Therefore, unless both methods are equally biased, the precision of visual counts was probably good. Zegers [*3*] also found a significant correlation between the number of ground squirrels visually counted above ground and the minimum number of ground squirrels known to be alive in a colony. He concluded that the accuracy of visual counts was good.

In this study, visual counts conducted over a period of at least three days produced the best correspondence to the mark-recapture population estimates. Because the highest counts were made in either the morning or afternoon peak activity periods, it would not be necessary in future studies to conduct visual counts throughout the day. Three or four counts a day made at 15-min to half-hour intervals during the peak activity period should produce a high degree of precision.

Visual counts should, however, be used with caution. Their precision is based

TABLE 1—*Comparison between the highest ground squirrel count for each day and the population estimate for the corresponding five-day period.*

Date	Time[a]	Population Estimate	Highest Count
6/14/79	0930	69	8
6/25/79	1500	77	20
6/26/79	1330	77	11
6/27/79	0825	77	31
6/30/79	1630	77	19
6/31/79	0815	77	22
7/15/79	1600	92	16
7/16/79	1700	92	17
7/17/79	0900	92	19
8/05/79	1100	104	14
8/06/79	1700	104	26
8/07/79	1630	104	19
8/08/79	0800	104	33
8/09/79	0905	104	38
8/25/79	1440	101	25
8/27/79	1500	101	27
8/28/79	1000	101	31
8/29/79	0850	101	38
9/18/79	0845	18	21
9/19/79	0900	18	23
4/15/80	1040	54	14
4/16/80	0715	54	29
4/17/80	1500	54	16
4/18/80	0730	54	27
4/19/80	1655	54	14
4/20/80	1500	54	39
4/21/80	0810	54	42
4/22/80	0745	54	35
5/03/80	0840	115	21
5/05/80	1230	115	30
5/06/80	0950	115	36
5/20/80	1700	58	33
5/21/80	0810	58	34
6/09/80	1630	66	40
6/10/80	0800	66	48
6/12/80	1700	66	37
6/13/80	0820	66	42
6/23/80	0930	140	54
6/24/80	1655	140	27
6/26/80	0700	140	54
7/15/80	1700	153	35
7/17/80	0800	153	44
8/03/80	0800	103	29
8/04/80	1600	103	30
8/05/80	0945	103	41
8/06/80	1125	103	30
8/24/80	0900	117	30
8/27/80	1400	117	38
8/28/80	0900	117	50
9/14/80	1300	6	6

[a]The time of day when the highest ground squirrel count was made is given.

TABLE 2—*Comparison between the population estimate (and 95% confidence interval) generated for each trapping period and the highest ground squirrel count during each period.*

Date	Population Estimate (95% Confidence Interval)	Highest Visual Count
10–14 June 1979	69 (60.2 to 77.8)	8
26–30 June	77 (66.2 to 87.8)	31
11–16 July	92 (72.4 to 111.6)	19
3–7 Aug.	104 (81.3 to 126.7)	38
23–27 Aug.	101 (86.3 to 115.7)	38
17–20 Sept.	18[a]	23
15–22 April 1980	54 (40.7 to 67.3)	42
29 April to 4 May	115 (96.2 to 133.8)	36
19–23 May	58 (51.0 to 65.0)	34
6–10 June	66 (51.3 to 80.7)	48
23–27 June	140 (110.4 to 169.6)	54
16–20 July	153 (123.2 to 182.8)	44
4–8 Aug.	103 (79.3 to 126.7)	41
25–29 Aug.	117 (92.2 to 141.8)	50
15–16 Sept.	6[a]	6

[a] Represents a total count of the population.

upon their repeatability. Therefore, as many factors as possible should remain constant between one count and the next. The same observer should conduct the counts during the pretreatment and posttreatment periods. The area to be counted should be predetermined and its boundaries well marked so that ground squirrels outside the area will not be counted. The area should be small enough (1 or 2 ha) that ground squirrels can be easily observed.

Typically, visual counts have been conducted for predetermined time periods (often 10 min). However, systematic scans of an area lasting variable lengths of time may be preferred because they minimize the possibility of counting squirrels more than once. In this study, scans were systematic and continuous (if no squirrels were observed in a portion of the study area, the scan was continued). No portion of the study area was scanned more than once. The only individuals counted twice were those that moved across the study area during the scan. By individually identifying animals during each visual count, I was able to quantify the amount of error; the error of overestimation produced by counting individuals more than once was 2.5%. The error was probably greater in this study than it would be in a normal field test because individual identification of squirrels lengthened the time needed to complete a scan and allowed greater ground squirrel movement.

A good study design is not enough to guarantee the precision of visual counts. A thorough knowledge of the life history of the animal being censused is necessary to avoid conducting counts during periods of low activity. Enormous seasonal variation existed in the percentage of ground squirrels active during the two-year study. Individual ground squirrels were active above

ground for only a short period (three or four months) during the year [7]. Emergence from hibernation occurred in late March or early April, with adult males emerging one to three weeks before adult females and yearlings. Breeding occurred immediately after emergence, with juveniles born in early May and appearing above ground in late May or June [7]. Adults hibernated in July and juveniles in September. The total population attained its maximum size between mid-July and August when adults were still above ground and the number of juveniles trapped was high. Adult and yearling populations were highest during May, and the maximum numbers of juveniles were present during late August.

Of those active, the percentage of animals above ground at any one time was also dependent upon the season. The season affected the length of the daily activity pattern of Richardson's ground squirrels. The percentage of time adults were active above ground during the day increased after emergence from hibernation until midsummer and then decreased until hibernation [*11, 12*]. The percentage of time above ground was low in early May, when the females spent a large proportion of time under ground while lactating, then increased to its highest level in June when the adults spent increasingly large amounts of time above ground feeding in preparation for hibernation [7]. The percentage of time spent above ground decreased again by mid-July [*12*] when the adults spent more time below ground as hibernation approached [*7, 13, 14*]. Juveniles spent little time above ground when they first emerged from their natal burrows in June; the percentage of time above ground was greatest in July and August and then decreased prior to hibernation in September.

During any evaluation of the efficacy of a control program, the pesticide treatment should occur when the population would normally be stable during the time between the pretreatment and posttreatment censuses. Because of the seasonal variation in the number of active ground squirrels and in the percentage of time individuals spent above ground, visual counts of ground squirrels should be avoided during certain times of the year: (1) when squirrels first emerge from hibernation, because individuals may emerge over a period of several weeks, (2) immediately after females give birth, because home ranges and aboveground activity are reduced, and (3) when squirrels begin to enter hibernation in midsummer, because both the number of individuals active and the percentage of time active individuals spend above ground decline.

The percentage of individuals present above ground was dependent upon the time of day. Ground squirrels exhibited a bimodal daily activity period during most of the year, with a peak period of activity in the early morning between 8 and 10 a.m. and a second slightly smaller peak in the late afternoon between 3 and 5 p.m. [7]. Ground squirrels did not appear above ground during midday [*7, 12, 15, 16*]. Visual counts should be conducted during these time periods; the survey time should not change between pretreatment and posttreatment.

Weather also affected ground squirrel activity patterns. According to Zegers [*13*], light intensity and air temperature were the most important factors ac-

counting for the number of ground squirrels above ground at one time. In June, cloud cover and air temperature were both influential in determining activity levels [*16*]. However, activity was not affected by air temperature or cloud cover early in the spring and summer or after mid-July [*11*]. According to Quanstrom [*17*], activity corresponded with times of most favorable air temperatures. Heavy snow, rain, hail, or temperatures above 80°F drove Richardson's ground squirrels below ground [*18*]. Therefore, visual counts should not be conducted during inclement weather, and the weather should be as similar as possible during pretreatment and posttreatment surveys.

Conclusions

Visual counting can be a valuable technique for estimating relative abundance of ground squirrels before and after a control program. The following are recommendations for the use of this technique:

1. Counts should be conducted on a predetermined, marked area of a size that is easily observed (1 or 2 ha).
2. Each count should consist of a systematic scan beginning at one edge of the study area and continuing to the other. No portion of the area should be scanned more than once.
3. Three or four scans should be conducted daily during the peak morning or afternoon activity period for three to five days.
4. Pretreatment counts should be replicated as closely as possible posttreatment by using the same observer and the same time periods.
5. Posttreatment counts should be conducted as soon as possible after the pretreatment counts (depending on the toxicant used) to minimize changes in the population not caused by the toxicant.

Acknowledgments

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Richard M. Anthony[1] *and Victor G. Barnes, Jr.*[1]

Plot Occupancy for Indicating Pocket Gopher Abundance and Conifer Damage

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ABSTRACT: Pocket gopher (*Thomomys mazama*) sign (mounds and earth plugs) was inventoried at 48-h and one-month intervals in study areas in central and south-central Oregon. Similar counts of 48-h and three-month accumulations of sign made by *T. mazama* and *T. talpoides* were taken in California, Idaho, and Oregon. Plot occupancy—the frequency at which sign occurred on circular, 81-m^2 plots—was strongly correlated with counts of individual signs and gopher depredation of planted conifer seedlings. For one area, we also determined that plot occupancy was significantly related to gopher density. We recommend the 48-h plot occupancy count—done in late summer or early fall, when gophers are most actively producing mounds—as the most useful index for forest management.

KEY WORDS: vertebrate pest control, conifer damage, population estimation, damage prediction, *Thomomys*

Pocket gophers (*Thomomys* spp.) pose a serious threat to successful regeneration of conifers in the western United States [*1–3*]. To plan and evaluate control practices effectively, foresters need a reliable and efficient method of determining the abundance of gophers. Population estimates of animals by direct count methods frequently are necessary for some investigations. However, an index of abundance can have as much value when measuring the impact of an animal on a resource, and it is usually less difficult to obtain. This is particularly true of pocket gophers, whose solitary and fossorial habits make capture time-consuming and visual counts of animals impractical.

[1]Zoologist and wildlife biologist, respectively, U.S. Fish and Wildlife Service, Denver Wildlife Research Center Field Station, Bend, Ore. 97701.

During snow-free periods, pocket gophers make two types of sign—mounds and earth plugs—that can be readily inventoried. Mounds are piles of soil deposited by gophers on the surface of the ground, whereas earth plugs are quantities of soil, often less than 8 cm in diameter and level with the ground, which gophers use to close openings in their burrow systems [*4*].

Several investigators have used counts of gopher sign to estimate abundance [*5*], but we are aware of only three studies that clearly demonstrate the validity of using sign counts as indexes. Despite conflicting results from sign counts for 48-h and 72-h periods, both Richens [*6*] and Reid et al [*4*] concluded that gopher density was highly correlated with counts of mounds. Scrivner and Smith [*7*], following the sampling schedule recommended by Reid et al [*4*], found a strong relationship between counts of sign and numbers of animals captured within strip transects.

In the present study, we evaluated plot occupancy—the frequency with which pocket gopher mounds and plugs occur in sample plots—as a technique for estimating pocket gopher abundance. Our objective was to determine if plot occupancy was related to animal density, counts of sign, and damage to conifer seedlings on forest plantations.

The study involved two phases. From 1969 to 1976 we developed and modified inventory procedures while conducting studies of pocket gopher/reforestation interactions in central Oregon. Similar studies conducted from 1976 to 1981 permitted an evaluation of the technique over a wide geographical area (California, Idaho, and Oregon).

Study Areas and Methods

Phase One: Determining Sampling Procedures (1969–1976)

Gopher populations and conifer damage were sampled on four study units each at Dugout Lake burn and Cave Mountain burn in central and south-central Oregon, respectively; the units ranged from 4 to 8 ha. Dugout Lake burn is 17 km west of Sisters, Ore., and Cave Mountain burn is about 6 km northeast of Chiloquin, Ore. The Mazama pocket gopher (*T. mazama*) inhabited each study area. The Dugout Lake plots were located on a lodgepole pine (*Pinus contorta*) plantation; the Cave Mountain site was planted with ponderosa pine (*P. ponderosa*).

Pocket gopher sign was inventoried on circular, 81-m^2 plots (about 5-m radius) with centers spaced 15 to 20 m apart. From 10 to 13 plots were located in lines randomly distributed throughout each study unit. Ten percent of the total area of each study unit was sampled with these plots.

To initiate sampling, all gopher sign was erased by leveling mounds and scraping soil over plugs. Plots were revisited at 48-h and one-month intervals and sign was counted. Earth plugs were recorded only for the 48-h interval because they tended to lose form after a few days of weathering. Mounds, which

deteriorated slowly, were tallied in each visit. These count data were converted to signs or mounds per hectare. Each plot also was recorded as either occupied by at least one sign or unoccupied. The percentage of occupied plots was used in data analysis.

Forty-eight-hour counts were conducted in each month from May (Cave Mountain) or June (Dugout Lake) through September. Sign recorded at the beginning of 48-h counts in August at Cave Mountain and September at Dugout Lake provided one-month data. There were 115 48-h counts and 43 one-month counts made during the eight-year study period.

Estimates of pocket gopher density were obtained only for the Dugout Lake site. There, two to three 0.2-ha (40- by 50-m) blocks were randomly located on each study unit and saturated with kill traps. Trapping was done in three separate years on appropriate study units so as not to influence gopher depredation on seedlings.

Pocket gopher damage to ponderosa and lodgepole pine seedlings was monitored for up to two years after seedlings were planted on randomly located lines of 10, 20, or 25 consecutive trees. Sampling intensity was about 5%, based on a stocking level of 1000 seedlings per hectare. In the analysis of damage data, the percentage of gopher-caused seedling mortality that accumulated from one spring to the next (one year) was compared with inventory data from the intervening month, when sign was most abundant (August or September). We believe the use of these measures was reasonable because damage by gophers primarily occurred from late fall to spring and usually resulted in seedling mortality.

Phase Two: Evaluation in Four Forest Communities in the Western United States

The opportunity to test the validity of our initial findings arose in 1976 when we initiated five-year damage control experiments in California, Idaho, and Oregon. Concurrent with these experiments, we inventoried gopher sign and recorded seedling damage to three conifer species in four areas: ponderosa pine on the Boise National Forest in western Idaho; Shasta red fir (*Abies magnifica* var. *shastensis*) on the Klamath National Forest in northern California; and lodgepole pine on the Deschutes National Forest in Central Oregon and the Targhee National Forest in eastern Idaho. *Thomomys talpoides* occupied the two Idaho sites; *T. mazama* occupied the California and Oregon sites.

Analysis of data from Phase 1 showed that a 5% sample by area provided acceptable precision—estimates within 20% of the population mean at $P = 0.05$. Therefore, we sampled twenty 81-m^2, circular subplots on each of four 3.2-ha study plots at each area each year. In August or September, we counted and obliterated mounds that had accumulated on subplots since the snow had melted (about three months). Then we revisited subplots to record 48-h accumulations of mounds and plugs.

Each study plot also contained 160 staked seedlings, which were distributed randomly in groups of four, to assess tree damage. Damage data collected after the second year were excluded from analysis because high seedling mortality reduced sampling intensity to below acceptable levels after this time.

Data Analysis

Data from Phase 1 and Phase 2 were subjected to regression analysis to describe relationships between plot occupancy and sign counts, plot occupancy and gopher density, and plot occupancy and seedling mortality. Logarithmic transformations were performed on sign count data before we analyzed plot occupancy and sign count relationships.

Results

Phase 1

Regression analysis of 48-h count data revealed significant relationships between amount of sign counted and plot occupancy. High correlations ($r \geq 0.80$, $P < 0.01$) were obtained for all months even though amount of sign made by gophers increased markedly from spring through summer. At Dugout Lake, the mean number of signs per hectare ranged from 53 in July to 342 in September; counts at Cave Mountain were lowest in May (56 signs per hectare) and reached a peak in August (272 signs per hectare).

Increased mound-building activity was primarily responsible for high sign counts in late summer. Because mounds comprised 73% of the sign in peak months, we compared the numbers of mounds with plot occupancy (based only on mounds) for 48-h periods. High correlations ($P < 0.01$) were obtained for Cave Mountain data in August ($r = 0.96$) and Dugout Lake data in September ($r = 0.90$).

At Cave Mountain, the plot occupancy and sign per hectare data for 48-h and one-month periods (all months combined) were significantly related ($P < 0.01$) and were best described by fourth-degree polynomial equations (Figs. 1 and 2). Fourth- and seventh-degree equations described those relationships for 48-h ($r = 0.99$, $P < 0.01$) and one-month ($r = 0.99$, $P < 0.01$) intervals at Dugout Lake.

Correlations between pocket gopher density and sign inventories at Dugout Lake were determined by comparing mean captures per trap block with the September sign count that immediately preceded trapping. The analysis showed that animal density was highly correlated ($P < 0.01$) with plot occupancy and signs per hectare for both 48-h ($r = 0.99$) and one-month periods ($r = 0.95$, Fig. 3).

Gopher sign inventories in both areas were reliable indicators of gopher depredation on pine seedlings. Although significant correlations ($r = 0.76$ to 0.99, $P < 0.05$) were found for plot occupancy or sign counts at 48-h and one-

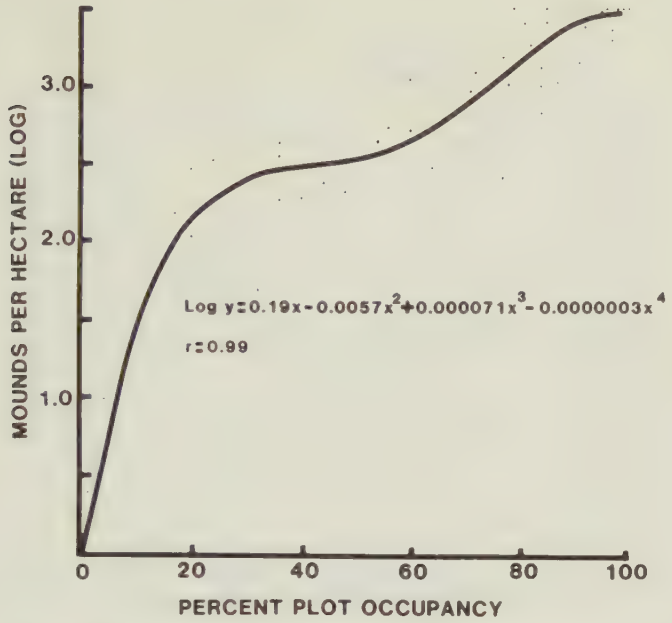

FIG. 1—*Relationship of the percentage of plot occupancy to the logarithm of the 48-h count of mounds at the Cave Mountain study area.*

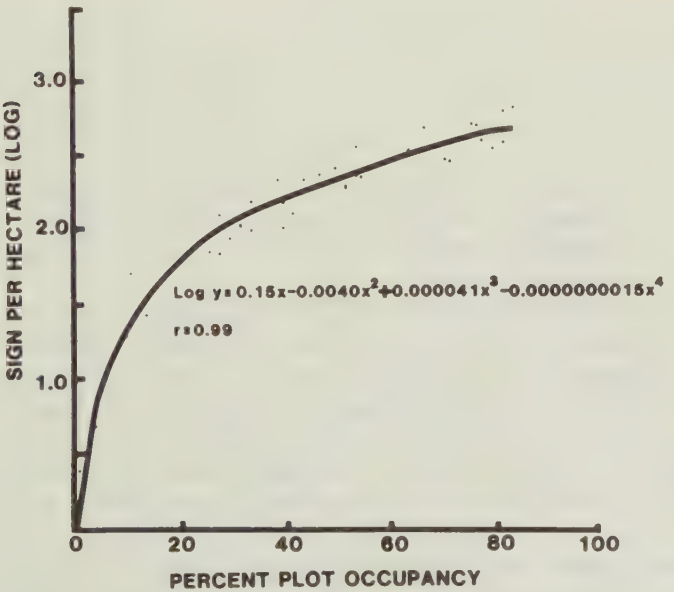

FIG. 2—*Relationship of the percentage of plot occupancy to the logarithm of the one-month count of mounds at the Cave Mountain study area.*

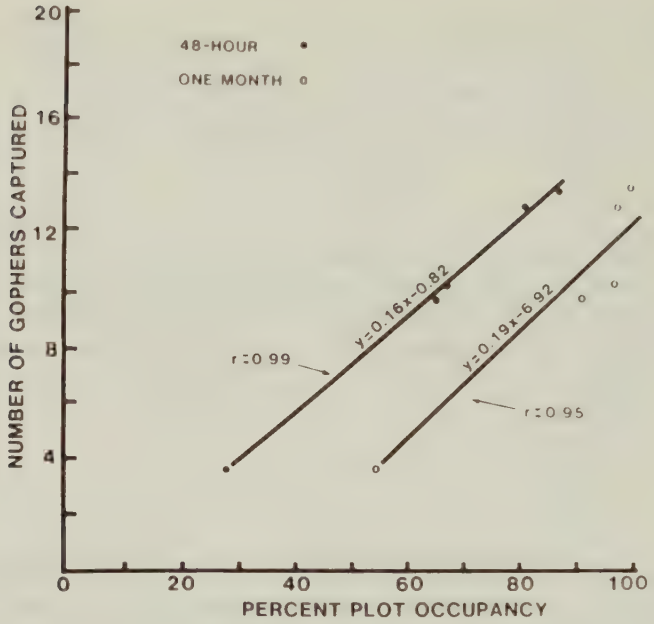

FIG. 3—*Relationships of the percentage of plot occupancy for 48-h and one-month intervals to the number of gophers captured on 0.2-ha blocks at the Dugout Lake study area.*

month intervals, the best relationship was between seedling damage and plot occupancy in 48 h (Fig. 4).

Phase 2

Correlations between number of mounds (which comprised 76% of all sign) and plot occupancy were significant ($P < 0.01$) for 48-h and three-month intervals at all four study areas. Correlation coefficients were highest for 48-h count data ($r = 0.87$ to 0.96).

Significant linear regressions ($P < 0.05$) of seedling damage on number of mounds occurred for the 48-h time period at all locations ($r = 0.66$ to 0.92) except the Targhee unit. The slope of the lines describing these relationships was different for each area. Seedling damage and 48-h plot occupancy were related only on the Deschutes ($r = 0.85$, $P < 0.01$) and Klamath ($r = 0.80$, $P < 0.05$) units. Plot occupancy and damage were high on the Targhee unit (averaging 63 and 43%, respectively); on the Boise unit they were low (25 and 4%, respectively).

Relationships between three-month sign inventories and seedling damage were not significant ($P > 0.05$).

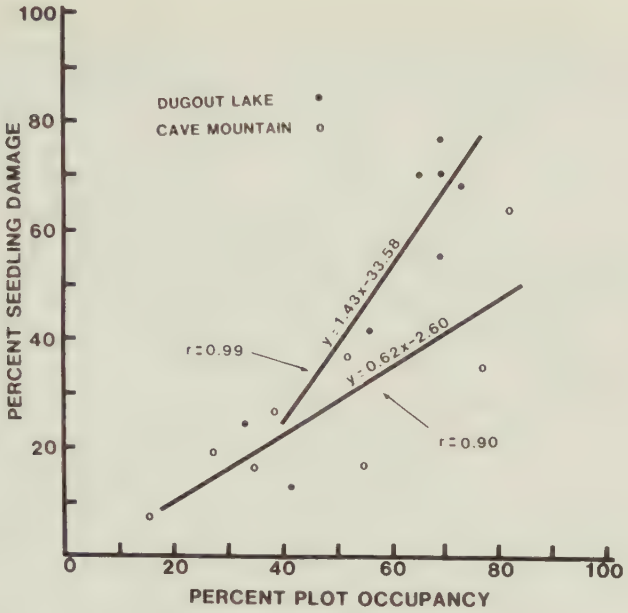

FIG. 4—*Relationship of the percentage of plot occupancy for 48-h intervals to conifer damage at the Dugout Lake and Cave Mountain study areas.*

Discussion

Results of this study indicate that use of sign inventories to monitor pocket gopher/reforestation interactions is valid. Other workers have shown that sign counts are positively related to gopher density [*4, 6, 7*], and our data from Dugout Lake support that conclusion. The strong correlations between plot occupancy and gopher density at Dugout Lake, and between plot occupancy and sign counts for all study areas, led us to conclude that plot occupancy inventories also provided reasonable indexes of gopher abundance.

Our data indicate that sign is a good indicator of seedling damage. Both plot occupancy and sign counts were strongly correlated with damage in Phase 1. Correlations between plot occupancy and seedling damage in Phase 2 were statistically significant for two of the four areas (the Deschutes and Klamath units); however, the premise of damage being related to plot occupancy held true for all four areas. On the Targhee unit where damage was extremely high, plot occupancy indicated high populations. Similarly, the Boise unit had very low damage and plot occupancy values. We suspect that the relationships were not statistically significant because the populations were at such extreme levels (high and low, respectively) that other site factors were a greater source of vari-

ation in damage than population. In addition, the sample taken in Phase 2 was probably inadequate for the heterogeneous distribution of gophers that can occur at low populations [8] and in less homogeneous habitats. Thus, for better precision in estimating damage from plot occupancy, we recommend using the larger sample size that was used in Phase 1 (that is, 10% by area).

Although both inventory measurements were dependable indicators of gopher abundance and depredation on pine seedlings, the plot occupancy procedure appears to be better suited for management application because it can be conducted with less effort and, therefore, at less expense. This advantage obviously would be greatest when indexing high populations.

Of the three time intervals that we tested (48-h, one-month, three-month), only the two shorter periods were satisfactory for estimating potential seedling damage. Overall, the 48-h inventory might be most useful because the information is quickly obtained and also because a 48-h census can be rescheduled several times and still be done during the period when gophers are most actively producing mounds. Thus, the resource manager can readily avoid work conflicts or repeat counts when adverse weather, livestock disturbance, or other factors bias results. The most meaningful information would result from consistent use of one time interval, regardless of which one is chosen.

Late summer or fall is the optimum time to estimate pocket gopher abundance on most forest lands of the western United States. That is when the rate of mound building tends to be greatest [5]. Also, it is an appropriate time to estimate potential tree damage because it immediately precedes winter when gophers are most destructive to conifer plantings [1]. Because earth plugs can be difficult to detect [6] and their omission does not affect the reliability of the estimate, we recommend that observers survey only mounds.

Our study indicates that the amount of damage caused by a given population of gophers can vary with habitat, probably because of site-specific factors such as snowpack characteristics, vegetative composition, and annual herbage production. Therefore, we recommend that land managers determine relationships between sign inventory and seedling damage for each habitat type where damage prediction is desired. This requires sampling of seedling damage as well as gopher sign. To obtain meaningful estimates of damage, we recommend annual examination and replacement of at least 50 staked seedlings per hectare (five rows of ten seedlings each). The sampling intensity that we recommend to inventory sign (plot occupancy) requires twelve 81-m^2 subplots per hectare. To simplify installation and reading of plots, representative 4-ha areas could be used to monitor larger reforestation units.

More information is needed to improve the accuracy of the plot occupancy sampling method. Plot size and population density can affect the sensitivity of this type of index [9]. A better understanding of how climatic and site factors influence mound building also is needed to aid in determining the best time for sampling. However, the method in its present form should greatly improve and expedite the land manager's ability to plan and evaluate control practices.

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Rameshwar P. Mathur[1] *and Ishwar Prakash*[1]

Methods Used in the Field Evaluation of Anticoagulant Rodenticides in India

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ABSTRACT: Field evaluation to establish the relative efficacy of three anticoagulant rodenticides for the control of desert rodents was made employing three census methods: active burrow counts, census baiting, and live trapping. The field testing was conducted in three phases: pretreatment population estimation, random placement of the candidate poison baits, and posttreatment population estimation. It is indicated that the burrow count method of population estimation is more reliable than the census baiting and live trapping methods.

KEY WORDS: vertebrate pest control, desert rodents, active burrow count, census baiting, live trapping, *Meriones hurrianae*, randomized block design, anticoagulants

Rodents are, by and large, the most destructive of vertebrates to the agricultural produce in India [*1*]. Chemical control is the only cheap, humane, and practicable method of rodent control, and among rodenticides, anticoagulants have been found effective against a large number of rodent pests [*2*]. Evaluation of the success of a rodenticide under field conditions has been a problem in many parts of the world, including India. Though a number of methods have been used to estimate populations [*3*], it is difficult to determine the actual number of animals. Hence, some observations before and after treatment are useful to determine the efficacy of a rodenticide in the field [*4–6*].

The present study was aimed at evaluating the relative efficacy of three anticoagulant rodenticides against a desert rodent population using three

[1]Research associate and professor, respectively, Coordinating and Monitoring Centre for Rodent Research and Training, Central Arid Zone Research Institute, Jodhpur 342 003, India.

methods (active burrow counts, census baiting, and live trapping) simultaneously on the same plots.

Procedures

Study Area

The study area was a scrub grassland of about 30 ha having scattered canopy in sandy plains of the Thar desert at Bikaner (latitude, 28°01′; longitude, 73°18′), Rajasthan, India. The farm is under the control of the Central Arid Zone Research Institute for range management experiments. The vegetation cover is mainly composed of perennial and annual grasses, a few herbs, and desert shrubs. Use of the area, a continuous stretch of land with almost homogeneous vegetation and infestation of rodents, facilitated comparison of methods for evaluating control success.

Four species of field rodents were trapped in the scrub grassland: the desert gerbil (*Meriones hurrianae*), the Indian gerbil (*Tatera indica indica*), the hairy-footed gerbil (*Gerbillus gleadowi*), and the sand-colored rat (*Rattus gleadowi*). However, *M. hurrianae* was found to be the predominant rodent species.

Layout of the Experimental Plots

The experimental areas were laid down following a randomized block design. In all, six blocks were selected. Each was divided into three plots of 1 ha, representing the three anticoagulant treatments; that is, there were six replicates of each of three treatments. A distance of 45 m between each plot and block was left as border area, so as to cover the average home range area of the rodents occupying the habitat and avoid crossovers between various treatment plots. Baits also were placed within the inner 90-m^2 area of each plot to further minimize the interference of rodents from other plots.

The bait stations were made of galvanized iron tubes (25 to 30 cm long with 7.5-cm diameters) available on the farm and used for nursery stock; they protected the bait from nontarget species. Pearl millet (*Pennisetum typhoides*) grain was used as the bait material, since it is preferred by desert rodents of Rajasthan. Arachis (*Arachis hypogea*) oil was used as an adhesive. About 200 to 300 g of bait material was placed in each bait station.

Methods for Assessing Efficacy

Three methods (burrow counts, census baiting, and live trapping) were used simultaneously on the same plots to determine the relative efficacy of the candidate poison baits. The trials were conducted in three phases: pretreatment census, poison baiting, and posttreatment census.

Burrow Counts—All the burrow openings, those occupied as well as those abandoned, were plugged with sand in the experimental area. The reopened burrows were counted early the next morning. Prakash et al [*7*] reported that during the first half hour after venturing out of their burrows in the morning the rodents prefer to feed; hence, burrow counts at this time usually represent single rodents. After completing the treatment and lag periods, the burrows were again plugged late in the evening and counted the next morning.

Census Baiting—Empty bait stations were placed in eight plots, four to five days before the pretreatment census was started for familiarization purposes. Ordinary pearl millet was placed in the bait stations for two days before and after treatment. The amount of bait consumed was measured to the nearest 0.1 g; the procedure was similar to that suggested by Rennison [*5*] and the European and Mediterranean Plant Protection Organization [*6*]. The bait stations with complete "takes" of the bait material were replenished with about double the quantity of bait.

Live Trapping—Sherman live traps were used with peanut butter as bait material. Since the census baiting method was also employed simultaneously in the same plots, the traps were placed away from the bait stations but randomly. In total, 90 traps were placed in six plots. Trapping continued for two days before and after treatment. Trap indices (rodents trapped per 100 traps in 24 h) were also calculated; but to determine the efficacy of a candidate rodenticide, the total numbers trapped before and after treatment were used [*4*]. The baits were replenished twice a day, since *M. hurrianae* is a diurnal rodent, whereas the other species (*T. indica, G. gleadowi*, and *R. gleadowi*) are nocturnal in habit.

Poison baits were placed at slightly different locations from the pre- and posttreatment census points to reduce the effect of prebaiting. The quantity of poison bait placed in the bait points corresponded to the maximum intake during the pretreatment census. The poison baiting was continued for ten days. A lag of four days was maintained between the termination of poison baiting and the start of posttreatment census, which allowed sick animals to die and affected individuals to recuperate. This period of 14 days (treatment + lag) corresponded with the laboratory evaluation of these poisons [*2*] during both no-choice and choice trials. All of the dead rodents were identified, sexed, weighed, and buried deep in the soil to minimize chances of secondary poisoning. The control percentages calculated by the three methods were compared by applying the test of analysis of variance.

Results and Discussion

Selection of a site with a mixed population of rodents was unavoidable, since in most of the habitats three or more species live sympatrically. However, the study site was clearly dominated by the Indian desert gerbil [*M. hurrianae* (Jerdon)].

The results revealed that the variation in the proportion of animals killed between the treatment blocks was not significant (Table 1*a*), indicating more or less uniform treatment in all areas. However, the percentage killed calculated was different with different census methods. Analysis of variance employed to determine the interaction between blocks, treatments, and methods indicated that interaction between treatments and methods was not significant (Table 1*b*). This further indicates that with any of the methods the efficacy trend of the poison remained the same. However, significant differences were found among the percentage killed calculated by the three methods. A comparatively high kill proportion was obtained with the burrow count method, which significantly ($P < 0.001$) differed from the results of the census bait and live trapping methods. However, between the latter two methods the difference was not significant (Table 1*b*). It is not possible to interpret whether burrow counting overestimates the control success or the other two methods underestimate it. However, a subsequent three-monthly monitoring of the population in the treated areas gave an indication that the control percentage calculated by burrow counting is more accurate. This method can be fairly applicable in other habitats, also, since many of the rodent species in India live a solitary life in their burrows [*1*], particularly during summer when their reproductive activity is minimal. Summer is also the recommended season for undertaking massive control operations [*1*]. The average number of rodents living in a burrow can also be calibrated for estimating the number of animals. However, in a mixed population of rodents the operator must be able to distinguish the burrows of various rodent species.

TABLE 1*a*—*Kill percentage as calculated by burrow counts, census baiting, and live trapping methods using three anticoagulant rodenticides.*

	Anticoagulant Treatments			
Methods	A	B	C	Mean
Burrow counts	90.5	83.2	81.1	84.9
Census baiting	73.0	62.6	61.0	65.5
Live trapping	75.0	73.6	58.6	65.7
Mean	79.5	72.4	66.9	72.9

TABLE 1*b*—*Analysis of variance of treatments and methods.*

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Sum of Squares	Variance Ratio	*P*
Methods	2	1224.82	612.41	38.27	<0.001
Treatments	2	353.39	176.69	11.04	<0.001
Treatments × methods	4	79.43	19.85	1.24	not significant
Error	22	352.11	16.00	...	...

In census baiting it is difficult to prevent nontarget species, especially the ants and birds, from consuming pre- and posttreatment census baits. A regular visit to the bait stations was found to be necessary, since at some places all the bait was taken away by the large colonies of ants. Some bait stations were filled with sand because of the sand-shoveling habit of the desert gerbils. The positions of such bait points were, therefore, slightly altered. Some workers [*8, 9*] consider food consumption as the most accurate method of population estimation, since very little interference is caused to the colony. This may be true for confined populations in a warehouse or in ricks; but in the field, baits can be consumed by nontarget species, and care has to be taken to prevent this. During the treatment period, most of the predominant rodent species are usually killed. The posttreatment bait consumption by the less common and socially inferior rodents is thus increased. This is evidenced by the posttreatment trapping results when *R. gleadowi* (75%), *G. gleadowi* (12.5%), and *T. indica* (12.5%) were trapped. *M. hurrianae*, which were found to be predominant in the pretreatment period, were not captured at all. This, along with the increased consumption by the nontarget species [a few babblers (*Turdoides caudatus*) also died from feeding on poison baits in the treated areas], may become a source of error in evaluating control success by showing low kill percentages.

An operator must be cautious when using the census baiting method with rodents that hoard bait material in their burrows, since this may introduce error in computing the "index reduction."

The removal method using traps was considered suitable, because using traps in the capture, mark, and recapture method requires a completely isolated test site. Moreover, a number of assumptions are to be made between the two trapping periods, such as the absence of differential reproduction, mortality, natality, and emigration and immigration occurring between marked and unmarked individuals [*8*].

Trapping is also useful in determining the species' composition and relative occurrence and the physical and reproductive conditions of the individuals inhabiting the area. However, for determining the efficacy of a candidate rodenticide, much depends on the trappability of the rodents, efficiency of the traps, activity of the animals, and so on. Moreover, behaviors such as trap shyness and neophobia also bias the study [*1*].

Conclusions

Considerable variance in the population estimates corresponding to the efficacy evaluation of anticoagulants utilizing the three methods was observed. Of the three methods used, burrow counts were found to indicate a higher level of control than the census baiting and live trapping methods. Another advantage of the recommended method is its applicability in other habitats and to other field rodents.

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Predators

Daniel B. Fagre,[1] *Walter E. Howard,*[1] *Douglas A. Barnum,*[1]
Roy Teranishi,[2] *Thomas H. Schultz,*[2] *and Donald J. Stern*[2]

Criteria for the Development of Coyote Lures

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ABSTRACT: The behavioral responses that coyote lures must elicit to attract coyotes to control devices and the chemical characteristics of lures that are required for practical field use are described. These criteria are being applied to the development of a new lure, designated the W-U lure. The first phase involves isolation, identification, and bioassay of key attractants found in coyote urine and anal sacs. Some of these chemicals have been formulated into the W-U lure, which elicits a greater degree of response from captive coyotes than any other synthetic lure and all but one commercial lure tested to date. The second phase of lure development entails standardized evaluations of the W-U lure by comparing it with other attractants in the field using a modified scent station survey. Distributing the W-U lure to field personnel responsible for controlling coyote damage also provides invaluable feedback, but standardized comparisons cannot be made. Preliminary results from both sources of field evaluation indicate that the W-U lure can contribute to improving coyote management.

KEY WORDS: vertebrate pest control, coyote, lure, odor attractant

Odorous substances have been widely used to lure coyotes to traps since the beginning of fur harvesting and predator control. The use of traps with odor attractants is still part of most control efforts to reduce coyote depredation of livestock to acceptable levels [*1*]. Western rangelands are the traditional sites of coyote conflicts, but coyotes continue to extend their geographic distribution [*2*] and, increasingly, are moving into new habitats such as urban areas

[1]Postdoctoral fellow, professor and vertebrate ecologist, and postdoctoral fellow, respectively, Wildlife and Fisheries Biology, University of California, Davis, Calif. 95616.

[2]Research leader and research chemists, respectively, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, Calif. 94710.

[3,4]. Thus, the need for effective coyote management is likely to increase. With public sentiment largely opposed to renewing the use of toxicants in baits [5], control techniques requiring odor attractants (traps and M-44s[3]) will remain in common use.

In spite of man's dependence upon odor lures to attract coyotes to traps and M-44s and the frequency of their use, there is little detailed information concerning the chemical and biological bases for attracting coyotes. Most coyote lures are empirically derived and categorized by the general behavioral responses they are intended to elicit (sex lures, food lures, and so on). Because many lures are fermentation products which have not been chemically studied, the chemical nature of these coyote lures is unknown. Statistical comparisons of lures for efficacy in the field [6–8] and the synthesis of the equivalent of an effective coyote lure [9] have contributed to improved use of existing lures. While these lures and the experientially gained knowledge for using them have occasionally led to efficient control of coyotes, the underlying principles for formulating lures require more attention.

Identifying the key chemicals in substances attractive to coyotes and increasing coyote behavioral responses with systematic changes in the combination and concentration of these chemicals might lead to the creation of synthetic, more effective odor attractants. Moreover, inherent batch-to-batch variation in the efficacy of some organic lures, difficulty in obtaining lure ingredients, and time and expertise required to age some lures could all be circumvented by synthesizing effective coyote lures. Because the odor quality of a synthetic lure is consistent, differences in its effectiveness can be ascribed to factors other than the lure itself. This may help to further identify environmental variables and trapping techniques affecting control success. Also, identification of specific chemicals attractive to coyotes may lead to the development of more coyote-specific lures. This development could decrease the take of nontarget species, both increasing the efficiency of coyote control programs and making predator management more acceptable to the public. The improvement of coyote odor attractants could contribute immediately to coyote management because lures are not subject to registration or other governmental restrictions.

Establishing Criteria for Coyote Lures

Criteria for the systematic evaluation of coyote lures for effectiveness can be divided into the coyote behavior-eliciting properties and the chemical characteristics required for practical use. For experimental purposes prior to field evaluation, additional criteria are needed to quantify captive coyote responses to lures. This allows ranking of candidate lures by their relative attractiveness;

[3]When the baited capsule holder of an M-44 placed at ground level is pulled by a coyote, the spring ejector propels sodium cyanide into the coyote's mouth.

only the most promising are subsequently field tested. We have used the amount of time, during 10-min tests, that captive coyotes spent investigating an odor attractant absorbed on a wool carpet piece at a scent station as a measure of its attractiveness [*12*]. However, the ultimate criterion of lure efficacy is its effectiveness in reducing depredations by free-ranging coyotes.

An ideal coyote lure should have as many as possible of the following properties.

Behavioral Properties

1. The lure should be detected by local coyotes soon after it is placed in the field. Therefore, the lure should be volatile enough for effective dispersal by air currents.
2. The lure should have a low detection threshold so that coyotes may detect and respond to it even at low concentrations amid various background odors.
3. Once detected, the lure must be of such compelling interest to the coyote that it interrupts other activities in which the coyote is engaged and elicits an approach to the control site.
4. The lure should be attractive at various concentrations. When used with expertise, the lure needs to be detectable by coyotes for only a few metres to draw them to a control device. A lure sufficiently strong to attract coyotes from a greater distance, however, may be too strong to investigate closely at the control site. A larger quantity of the lure or a more concentrated preparation can be dispersed nearby to draw coyotes from much greater distances. Once attracted to the area by the diffused odor, coyotes should be able to readily locate the odor source at the control device.
5. The lure should sufficiently compel investigative behavior to overcome the coyote's normal caution and ensure interaction with the control device.
6. Ideally, the lure should elicit specific behaviors such as a bite-tug response for use with the M-44.
7. The lure should be species specific, attracting only coyotes.
8. The lure should have biological importance to coyotes, containing compounds similar to those in food items, sex pheromones, or other substances to which coyotes are strongly attuned. This will reduce neophobia as the coyote encounters the odor and may reduce association of the odor with humans. Also, this will ensure that the odor has durable attractant qualities, that is, that coyotes do not respond to it only as a novelty.
9. In general, the lure should be attractive to all coyotes, irrespective of sex or age, and there should be little individual variation in their responses to it. However, a variation of the lure that is selective for a given sex or age class may also be valuable in some management situations.
10. The lure should be highly attractive to coyotes during all seasons.
11. The attractiveness of the lure should not vary significantly with temper-

ature, relative humidity, atmospheric pressure, or regional habitat differences (such as the food base for the coyotes) if it is to be consistently effective.

Chemical Characteristics

12. The chemicals in the lure should be nontoxic, noncorrosive, and safe for both humans and domestic animals, whether contacted dermally or ingested.
13. The lure should have a small number of ingredients, which are readily available and inexpensive.
14. The lure should be easily prepared and not require expensive equipment to produce or store safely. It should be useable immediately after being prepared. It should also be acceptable for shipping by mail.
15. The lure's volatility should be low enough so as to remain detectable for a week or whatever the interval is between visits to the control site by the personnel responsible for controlling damage.
16. The lure should not react readily with chemicals it will contact in the field and should not degrade quickly with exposure to sun, air, or moisture or under storage conditions.
17. It should, however, be biodegradable, breaking down after a few weeks or months in the field.
18. The lure should not translocate in the environment (soil/water) or concentrate in animal tissues.
19. It should be inert enough to be readily mixed with traditional lure materials (fats, meat, blood, urine).
20. The lure should be effective in small doses; one to a few drops would be ideal. Some coyotes may respond neophobically if the attractant material is too visible.
21. The lure should be easily prepared in a form appropriate for use with a variety of control methods without requiring special procedures to transport it in vehicles or to apply it in the field.
22. The lure should not be so offensive that personnel responsible for controlling coyote damage are unwilling to use it.
23. The materials constituting the lure should not be under a plethora of regulatory restrictions or present political difficulties in obtaining and using them (such as whale oil).

Using Criteria to Develop a Coyote Lure

Two phases of research are necessary for developing lures that might satisfy most of these criteria. At first, captive coyote studies provide immediate and relatively inexpensive information on inherent coyote odor preferences. It is possible to quickly screen candidate attractants, quantify coyote behavioral responses, and rank the relative attractiveness of formulations in large numbers. However, these studies do not assess the behavioral responses of free-

ranging coyotes to attractants. Thus, the second phase is to confirm the attractiveness of the most promising lures by systematic exposure to coyote populations in the field.

In the first phase of lure development, vertebrate ecologists direct chemists to odorous materials attractive to coyotes. The chemists then identify the chemical structures of these materials and furnish fractions of the material [*10*] for testing on captive coyotes [*11*]. Further fractions and single compounds are subsequently tested until at least some of the key biologically active components are identified [*12*]. With the chemical basis for attractiveness partially established, the two groups of scientists can then cooperate to establish the biological bases for the attractiveness of the chemicals identified by noting where they are found in biological systems and what significance they may have for coyotes. In addition, they need to document how specific coyote behaviors vary when systematic changes are made in the chemical composition of the odors. The behavioral responses of coyotes to odor attractants are more easily studied in detail in captivity than in the field. Pen studies can also be used to identify many other pertinent factors in developing effective coyote lures, such as the effect of ambient temperature on a compound's attractiveness to coyotes. The goal is to synthesize the optimum combinations of compounds and concentrations which will evoke the greatest response from coyotes. Ideally, specific behavioral responses will also be correlated with specific compounds.

The second phase of coyote lure development is to formulate and field test the most promising lures and, eventually, deploy them in actual damage control operations. It is difficult to assess the relative efficacy of lures based on their use by field personnel because each person has an individual style of trapping, and coyote populations vary in their vulnerability to control efforts. Therefore, standardized field tests to evaluate the relative attractiveness of candidate lures should be conducted before they are used in the field by personnel responsible for damage control. A scent station survey, used to monitor predator populations in 17 western states [*6*], has been modified to compare the efficacy of candidate lures in the field [*7,8*]. Using information from the first phase, the results of field tests should make it possible to refine the odor attractant or change other variables affecting the lure's efficacy in the field (such as using different carriers).

At least one effort other than that described in this paper has been made to systematically create a coyote lure of known chemical composition. The volatile, biologically active components of fermented egg product, an established canid attractant, were isolated and identified [*13*]. A human panel was then used to compare fermented egg product with a synthetic version (synthetic fermented egg or SFE) with the goal of using this consistently attractive coyote lure as a standard in the field [*9*]. High visitation rates by free-ranging coyotes for SFE have been reported [*8*]. Recently ten of the fatty acids in SFE were found to elicit as much response as SFE and other SFE variations [*14*]. Cap-

tive coyotes have also been strongly attracted to SFE but have also responded with significantly greater interest to some other available lures [*12*]. Some field personnel responsible for controlling coyote damage indicate that SFE is not as attractive as other available lures.

Development of W-U Lure

A review of the development of the W-U lure (patent pending by Western Regional Research Center, U.S. Department of Agriculture, and the University of California, Davis) shows that it satisfies many of the criteria for a superior coyote lure, pending further field testing.

In the first phase of our research program, the foremost task was to identify those chemicals that attract coyotes. We studied the potential canid chemical signals which are transmitted in various secretions or by depositing urine. Scent marking provides ecologically important information to conspecifics [*15,16*], and coyotes are probably sensitive to the chemical signals because of selective pressures. Coyote urine is the most universally used lure for trapping coyotes. Although the odors from coyote urine are apparently interesting to coyotes, not all of the chemicals in the urine are likely to be important attractants. Hence, the identification of the key attractive chemicals in urine was undertaken [*10*]. In a similar approach, biochemical analyses of anal sac secretions of coyotes [*17*] led to tests of some of the identified volatile fatty acids as potential coyote attractants [*18*].

Trimethylamine has been identified in the anal sacs of coyotes and dogs [*17*], in red fox anal sacs [*19*], and in domestic dog urine [*20*]. When this amine is combined with valeric acid, one of the constituents of coyote urine [*10*], the product is trimethylammonium valerate (TMAV) [*21*]. This salt of a C_5 fatty acid proved to be highly attractive to captive coyotes at the University of California Hopland Field Station [*12*]. TMAV releases trimethylamine slowly and, as an odor source, lasts many times longer than trimethylamine alone.

Because other organic acids could form salts with trimethylamine, an optimization study was conducted. Trimethylammonium decanoate (TMAD), a salt of a C_{10} fatty acid, proved to be 4.2 times more attractive than TMAV (Fig. 1). TMAD elicited robust responses by 20 captive coyotes in 93% of the trials ($N = 156$), held coyotes at a test station longer than any other synthetic attractant tested, and acted as an effective odor stimulus for as long as nine days in the odor-saturated environment of our coyote colony at Hopland. TMAD also elicited a high proportion of licking and biting in the behavioral repertoire of coyote odor responses (Fig. 2). This capability may be useful for increasing the efficiency of M-44s, the operation of which depends upon coyotes pulling the capsule holder.

We have tested hundreds of candidate attractants since 1972, and only one commercially available lure, Carman's Distant Call Lure (CDCL), had ever proven more attractive to captive coyotes than TMAD. This commercial lure

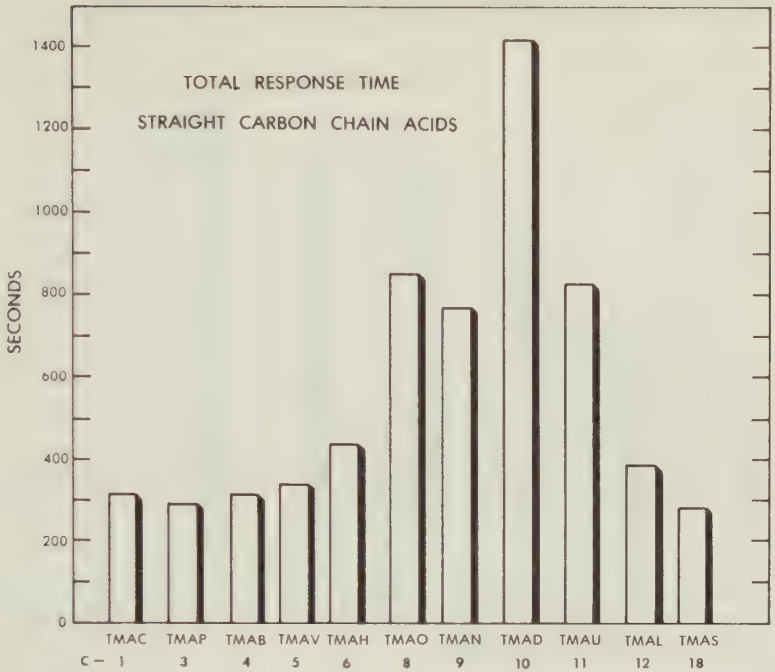

FIG. 1—*Time spent by ten adult captive coyotes (sexes equal) responding to trimethylamine (TMA) salts of organic acids from C_1 to C_{18}: C (carbonate), P (propionate), B (butyrate), V (valerate), H (hexanoate), O (octanoate), N (nonanoate), D (decanoate), U (undecanoate), L (laurate), S (stearate). Each coyote was singly tested twice with each odor for a 10-min period on different days.*

is of unknown chemical composition and is a complex product of many constituents.

A comparison was made between TMAD, CDCL, North Dakota lure (another complex natural lure), a coyote urine fraction, and our previous synthetic lure. They were the most attractive lures we had evaluated at Hopland at the time of this comparison (Fig. 3). The results indicate that TMAD is generally as attractive as the commercial lures used in this test, on one occasion better than the consistently most attractive lure (CDCL), and considerably better than the nonestrous acids fraction from coyote urine or TMAV with ethylbutyl sulfide added (TMAV + EBS). Because trimethylamine has not been found in domestic cat anal sac secretions [*17*], TMAD may be more coyote specific in its attractant qualities than other lures. Tests are planned with other captive carnivore species to evaluate TMAD with regard to the species-specificity criterion and examine the types of behavioral responses TMAD elicits from other carnivores.

TMAD has many of the chemical properties required of an ideal coyote lure.

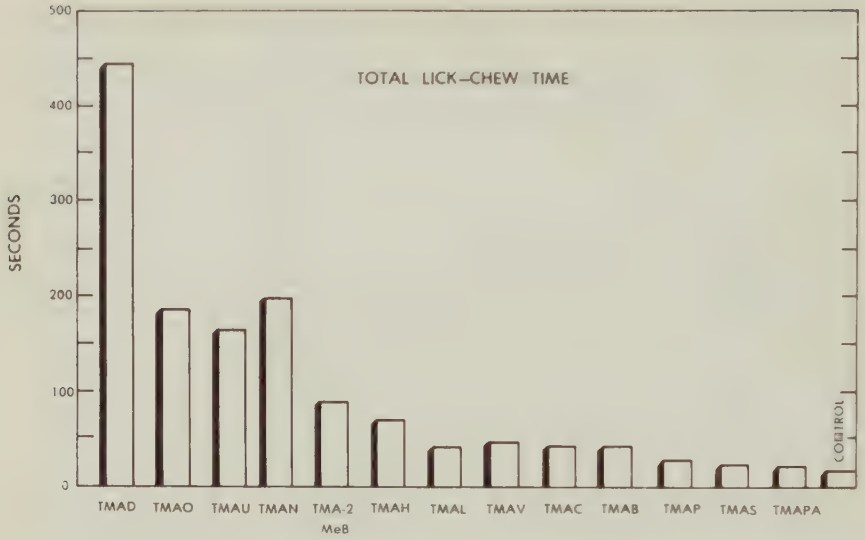

FIG. 2—*Total time spent licking and chewing by ten adult captive coyotes (sexes equal) in response to TMA salts of organic acids (as in Fig. 1), but with TMA 2-methylbutyrate (TMA-2 MeB) and TMA phenylacetate (TMAPA) added. Each coyote was singly tested twice with each odor for a 10-min period on different days.*

Because it is a salt of a weak base and weak acid, it is buffered. It is stable, not readily breaking down or reacting with other chemicals, and it releases trimethylamine slowly. Coyotes have responded to very small volumes of TMAD placed in the field. TMAD is currently regarded as a potentially effective lure with no toxic or environmentally damaging qualities, is inexpensive and simple to produce from readily available materials, and has consistent odor properties.

Many commercial coyote lures have a skunklike aroma, presumably due to the presence of various sulfur compounds. The sulfur compounds are present in mustelid anal sacs [*22*], which are used in formulating some lures. Because some of the "skunky" lures are attractive, and because sulfides have been identified in the neutrals fraction of coyote urine [*21*] and in red fox urine [*23*], we evaluated the change in attractiveness of TMAD when a selection of sulfide additives was combined with it (Fig. 4). The results from this test indicate that TMAD with the sulfide additives evokes greater response times from coyotes than TMAD alone. However, the lack of statistically significant increases in response times ($P > 0.05$) suggests that responses to TMAD are near the upper limit of coyote responses to odor attractants (Fig. 5). We cannot expect continual increases in response time with further improvements to a specific attractant because other environmental stimuli require a coyote's attention regardless of a lure's attractiveness. In fact, certain additives can detract from TMAD's attractiveness (Figs. 4 and 5).

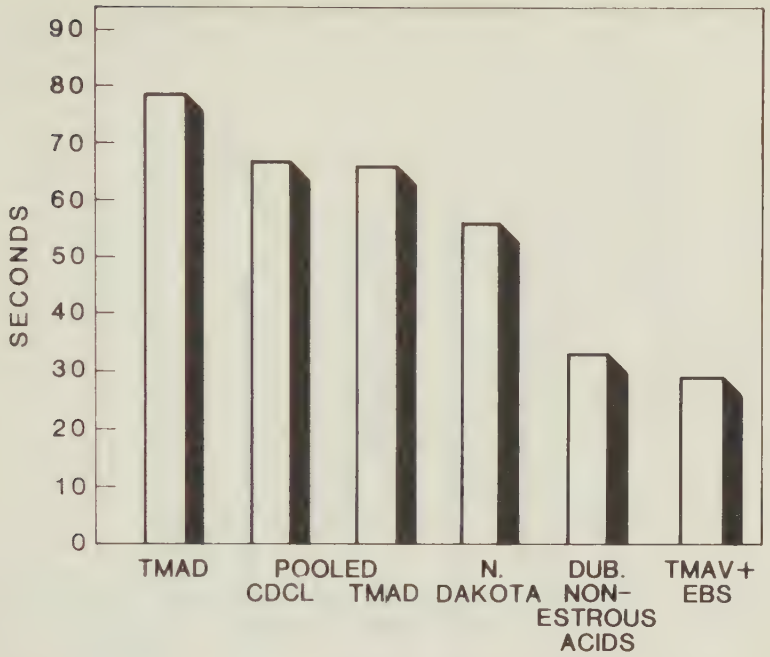

FIG. 3—*Comparison of mean response times per captive coyote exposure to trimethylammonium decanoate (TMAD) during peak sensitivity (N = 18 exposures, January 1981) with Carman's Distant Call Lure (CDCL) (N = 32 exposures, pooled from 1981 tests), TMAD (N = 32 exposures, pooled from 1981 tests), North Dakota lure (N = 12 exposures, December 1978), acids from nonestrous coyote urine collected in Dubois, Idaho (DUB) (N = 6 exposures, February 1979), and trimethylammonium valerate plus ethylbutyl sulfide (TMAV + EBS) (N = 20 exposures, March 1980). Between three and ten adult captive coyotes were singly tested twice with each odor for a 10-min period during these various tests.*

The results from these and other tests led to the synthesis of the W-U lure, which incorporates TMAD with several promising sulfide additives. Although the W-U lure seemed the best coyote attractant in early tests at Hopland, it is not an ideal lure which meets all the necessary criteria. One of the criteria not yet satisfied by the current W-U lure is year-round effectiveness. The responses of captive coyotes to TMAD indicate that the W-U lure is most effective during the winter breeding season (Fig. 6). The field use of the W-U lure by personnel responsible for coyote damage control tends to corroborate this seasonality of TMAD's attractiveness.

Preliminary Field Evaluation of W-U Lure

The second phase of using established criteria to develop lures is to evaluate the candidate attractant or attractants in the field. Procedures and techniques for standardized field evaluations of the relative attractiveness of coyote lures

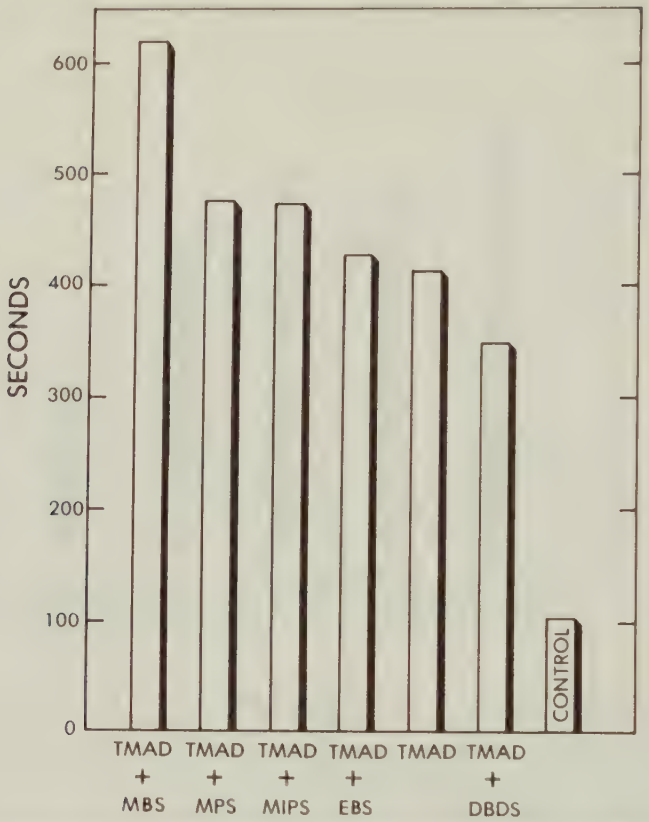

FIG. 4—*Comparison of time spent by ten adult captive coyotes (sexes equal) responding to TMAD with and without the following sulfide additives: methylbutyl sulfide (MBS), methylpropyl sulfide (MPS), methylisopentenyl sulfide (MIPS), ethylbutyl sulfide (EBS), and dibutyl disulfide (DBDS). The control is an untreated scent station. Each coyote was singly tested twice with each odor for a 10-min period on different days.*

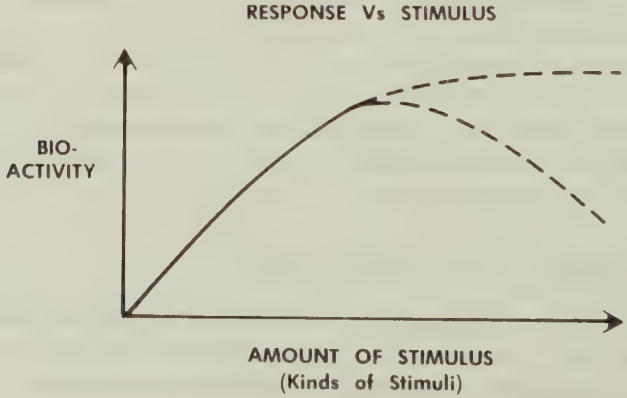

FIG. 5—*Bioactivity response versus amount of stimulus or kinds of stimuli.*

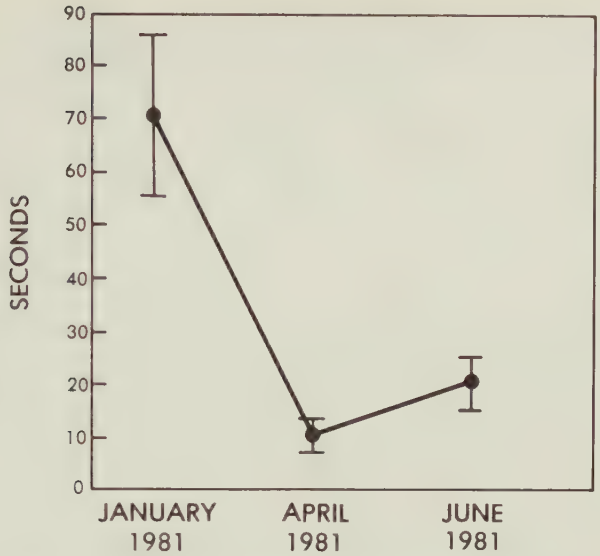

FIG. 6—*Mean coyote response time per exposure to TMAD during 10-min tests in January, April, and June 1981 by ten adult captive coyotes (sexes equal). Each coyote was singly tested twice with TMAD each month.*

have been described [*7,8,18*]. A limited scent station test compared the relative attractiveness of CDCL, SFE, and W-U lure from July through September 1981 on the Hopland Field Station in northern California (Table 1). It was not possible to continue this test into the winter when responsiveness to TMAD by captive coyotes is at its highest level. Nevertheless the three lures were considered to be roughly comparable in attractiveness. W-U lure was tested at a low concentration, which has since been found to be less effective than higher concentrations. Visitation rates by coyotes were also low throughout the test (1.3 to 1.8 visits per 100 exposure days). Thus, a comprehensive evaluation is still required to accurately rate the attractiveness of W-U lure to free-ranging coyotes.

The W-U lure has also been used by various personnel responsible for damage control in five western states on a trial basis and, while the results are strictly anecdotal to date, the W-U lure appears to be increasing trap and M-44 effectiveness. Plans are under way to obtain quantitative and qualitative data on the performance of W-U lure in actual damage control situations.

Conclusions

By applying criteria for an ideal coyote lure, and systematically analyzing and evaluating odor attractants, a team of chemists and wildlife ecologists can advance our understanding of coyote olfactory behavior and the chemical and biological bases for formulating effective lures.

TABLE 1—*Comparison of coyote visits to field scent stations[a] containing one of three coyote attractants, at Hopland Field Station, July through September 1981.*

	W-U Lure, cc[b]	CDCL, cc[c]	SFE, g[d]
Amount exposed	1	1	1
No. of scent stations	13	5	10
No. of exposure days	1076	236	728
No. of coyote visits	14	4	13
Visits per 100 exposure days	1.3	1.7	1.8

[a]Approximately 1-m circles of sifted dirt and sand were situated at 0.16-km intervals along fire breaks and dirt roads and checked five times weekly for evidence of coyote visits to the attractants placed in the center of the circles. W-U lure and CDCL were allowed to soak into pieces of rabbit fur staked to the ground (part of other research designed to increase biting responses), and SFE was contained in tissue capsules used in the U.S. Fish and Wildlife Service predator abundance surveys.

[b]A 0.1-ml amount of undiluted W-U lure (TMAD plus selected sulfides, see text for more information) was mixed with 454 g (1 lb) of lard. At this concentration W-U lure was easily detected by humans.

[c]Carman's Distant Call Lure (CDCL) was used as received from the supplier.

[d]Synthetic fermented egg (SFE) was obtained from a U.S. Fish and Wildlife Service scent station survey kit for the annual predator abundance survey. The SFE is adsorbed onto a polyamide resin powder used to fill the tissue capsules.

The development of the W-U lure exemplifies the type of results possible from such an approach. The W-U lure has a chemically established basis of attractiveness to coyotes, has biological significance for coyotes, has a known chemical composition which is easy to duplicate, has consistent odor properties, is safe to use, is relatively inexpensive, and has long-lasting attractiveness in the field. Although it is by no means the best potential lure that could be developed, it suggests the possibilities that await us in the effort to develop improved, sophisticated techniques for dealing with wildlife problems. With continued research, we may soon have a variety of coyote odor attractants which will collectively meet the criteria for ideal coyote lures.

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Peter J. Savarie[1] *and Guy E. Connolly*[1]

Criteria for the Selection and Development of Predacides

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ABSTRACT: Since 1940, the Denver Wildlife Research Center has been evaluating toxicants for the control of coyotes. These lethal agents for coyotes and other predators are commonly called predacides. This paper describes our current criteria for the selection and development of predacides. Efficacy standards under practical working conditions, human and environmental safety factors, and compliance with registration requirements established by the U.S. Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) are important criteria. Most of the criteria were developed for chemicals that would be used in the livestock protection collar (toxic collar), but they also apply to other predacidal techniques such as single lethal dose baits and large baits.

The first criterion is that the chemical be toxic to coyotes, although tests on coyotes may be precluded for chemicals that are hazardous to humans. Of prime importance is that the chemical not be dermally toxic or carcinogenic. An antidote is highly desirable but not essential. A predacide should be tasteless and odorless so that it is well accepted and should be effective at low oral doses so that the amount used is not excessive and bulky. A predacide should be effective within 24 h so that predation can be stopped as soon as possible. Chemicals that are economical and commercially available are highly desirable. Residue levels in poisoned coyotes should not be toxic to scavengers. For livestock-borne devices such as the toxic collar, the toxicant should not leave harmful residues of chemical in sheep exposed to sublethal doses. The presence of such residues could prevent marketing of sheep that had worn toxic collars. If the sheep dies, residues in or on the sheep should not be toxic to scavengers. Selective toxicity to coyotes is desirable so that nontarget species will not be poisoned if they come in contact with the chemical. Last, the chemical should be registerable and fulfill requirements as set forth by regulatory agencies.

KEY WORDS: vertebrate pest control, predacides, standard evaluations, criteria, acute oral toxicity, toxic collar, single lethal dose baits, Compound 1080, methomyl, carbofuran, sodium cyanide, diphacinone

[1]Research pharmacologist and research wildlife biologist, respectively, Denver Wildlife Research Center, U.S. Fish and Wildlife Service, Denver, Colo. 80225.

Toxic chemicals have been used to kill mammalian predators (such as coyotes, foxes, wolves) in the United States for at least 135 years. Toxicants for predators are commonly called predacides, and the first toxicant for this purpose, strychnine, was used as early as 1847 [*1*]. Strychnine alkaloid and strychnine sulfate have been used extensively to kill coyotes and many other mammals. Thallium sulfate [*2*] has also been used as a predacide but was discontinued in 1967 by the U.S. Fish and Wildlife Service (FWS) [*3*] because it was considered to be too hazardous. Sodium monofluoroacetate (Compound 1080) was used for coyote control from 1946 to 1972, but Executive Order No. 11643 [*4*] banned the use of all predacides in federal programs and on federal lands. The U.S. Environmental Protection Agency (EPA) then suspended interstate shipment and canceled registration of all chemicals for predator control. The executive order was modified in 1975 [*5*] and 1976 [*6*] to allow the use of sodium cyanide in a mechanical device known as the M-44, and currently sodium cyanide and a gas cartridge containing sodium nitrate and charcoal are the only predacides registered by the EPA.

In the 1950s and 1960s, Compound 1080, strychnine, and sodium cyanide were the most commonly used predacides. Cancellation of their use spawned efforts to find replacements that would be just as effective and "registerable" by federal and state regulatory agencies. In 1974 the Denver Wildlife Research Center of the FWS began research on chemicals [*7,8*] that could be used in the toxic collar, a technique developed by McBride [*9*] to deliver toxicant to coyotes as they attack sheep or goats. This research led to an EPA experimental use permit (No. 6704-EUP-14) under which the FWS evaluated Compound 1080 in these collars. The research culminated in submission by the FWS in September 1981 of an application to the EPA to register Compound 1080 for this purpose.

Since 1974 we have established criteria that serve as guidelines for the research and development of the "ideal" predacide. These criteria consider efficacy standards under practical working conditions, human and environmental safety factors, and regulations as established by the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Some criteria are specific to chemicals for the toxic collar, but most are applicable to other predacidal applications such as the M-44, small or large baits, licking stations, or toxic bait posts.

Methods

Procedures for maintenance of coyotes and conducting acute oral LD_{50} (lethal dose, in milligrams per kilogram, that kills 50% of a test population) tests have been described [*10*]. Toxic collars were tested with captive, penned coyotes and under field conditions [*7,8,11,12*]. Small baits were prepared with either edible beef tallow or a mixture of 90% beef tallow and 10% beeswax and

weighed about 9 g. Information about chemicals was obtained through computer searches of literature references and personal communication with representatives from private industry and other governmental agencies.

Criteria Used to Evaluate the Candidate Predacides

Low Oral Dose Effectiveness

It is important that predacides be potent to the target animal so that the bulk of the chemical does not cause formulation and delivery problems. The acute oral LD_{50} of Compound 1080 in coyotes is 0.12 mg/kg, and a solution of 10 mg/ml is adequate to achieve consistent mortality in the toxic collar. Only 5 mg is needed for a single-dose coyote bait [*13*]. In contrast, the acute oral LD_{50} of para-aminopropiophenone (PAPP) is 5.6 mg/kg, and 400 mg/ml is required in the collar and at least 150 mg in a single-dose bait. The acute oral LD_{50} for methomyl is 5.6 mg/kg, and collars must contain 400 mg/ml of it to kill attacking coyotes. The acute oral lethal dose is probably a good indicator of the actual dose needed for the collar, but it may be misleading when applied to baits. For example, tallow and meat baits containing 170 mg (about three LD_{50}s for a 10-kg coyote) of methomyl were not lethal to two coyotes [*14*]. Methomyl can be metabolized rapidly [*15*], and it appears that coyotes absorb it slowly from baits and detoxify it quickly.

Taste and Odor of Chemicals

Experience with a broad class of chemicals including organophosphates, carbamates, organofluorines, and anticoagulants indicates that chemicals with noxious taste or odor are likely to be rejected by coyotes in either bait or toxic collar formulations. A solution of 33% sodium cyanide in collars was used in penned tests, and 75% (nine out of twelve) of the coyotes that bit the collars died [*11*]. But it was noted that the solution had repellent properties because after receiving a dose coyotes frequently pawed at their mouths and rubbed their muzzles on the ground. This solution has a discernible odor, an alkaline pH of 14, and is caustic. Wild coyotes apparently were more sensitive to these properties; in field tests, eight collars were punctured, but no dead coyotes were recovered [*7*]. Lethal doses of sodium cyanide immobilize coyotes within 3 min [*11*], and dead coyotes should have been recovered near the site of the bitten collar. In contrast to the offensive taste and odor of sodium cyanide, the anticoagulant diphacinone is odorless and probably tasteless because rats eat baits containing it without developing an apparent bait-shyness. Coyotes readily attack and hold onto collars that contain it [*8*]. Compound 1080, methomyl, carbofuran, ethylene glycol, and PAPP are other chemicals that are well accepted by coyotes.

Speed of Predacide Action

The speed of action of predacides varies widely. Some are effective within a few minutes whereas others are delayed in action for several days [*7,8*]. Both methomyl and carbofuran can immobilize coyotes within 3 to 5 min [*14*], and death usually occurs within 1 h. The death times of three coyotes that punctured Compound 1080 toxic collars ranged from 2½ to 5¼ h [*8*], whereas diphacinone usually kills within 5 to 10 days but can take as long as 17 days [*10*]. One disadvantage of diphacinone in the toxic collar is that lethally dosed coyotes may continue to kill sheep before they succumb [*16*]. In the toxic collar, a fast-acting predacide is needed to stop further depredations by any coyote that attacks livestock. Moreover, a predacide that killed within minutes would permit recovery of poisoned coyotes near the scene of collar attacks. This would provide proof of efficacy and also permit proper disposal of poisoned carcasses. For other predacidal techniques, such as baiting to reduce coyote numbers on spring lambing ranges before livestock arrive there, slow-acting toxicants such as diphacinone may be acceptable.

Not Hazardous to Humans

Any chemical, including table salt, can be misused so as to cause harm to humans. But with common sense many chemicals can be safely handled and used as predacides. In screening new chemicals, we avoid any that have high dermal toxicity or are known or highly suspect of being carcinogens. For example, nicotine could be a desirable predacide because it acts rapidly and coyotes would be expected to be found within a few feet of the site of contact with it. But nicotine is absorbed from the respiratory and gastrointestinal tracts and intact skin [*17,18*]. As little as 60 mg (1 mg/kg) or less can be fatal to an adult human. Therefore, we have not seriously considered nicotine for predacidal applications. Metabolites of the fungus *Aspergillus flavus* called aflatoxins are highly toxic but are also some of the most potent carcinogens known [*19*]. Methomyl and carbofuran, carbamate insecticides that are widely used, have low dermal toxicity [*18*] and have been evaluated as predacides with some success. High acute oral toxicity *per se* does not automatically rule out the use of a chemical, because some highly acute toxicants such as Compound 1080 have proved through their historic use not to pose a hazard to humans.

Antidote or Statement of Practical Treatment

An antidote is highly desirable but not an absolute necessity because several registered vertebrate pesticides do not have antidotes. Zinc phosphide is an acute rodenticide that does not have an antidote, but it does have a statement of practical treatment to alleviate poisoning symptoms. Many

commercial products that lack antidotes do have effective first aid and emergency treatment procedures [*17*].

Environmental Safety

Selective toxicity to the target animal is desirable to prevent poisoning of nontarget animals. However, the mode of application has equal or greater influence on nontarget hazards. With the toxic collar, for example, exposure to nontarget animals is minimal [*7,8*]. Exposure of nontarget animals to toxic baits presents a greater hazard, so the need for selective toxicity is probably greater for baits than for toxic collars. Nontarget poisoning has not been observed with the use of toxic collars, but it has been reported for bait stations [*2*]. The accumulation of residues in vegetation and soil is an important consideration in assessing potential environmental impacts, but all the evidence indicates that it does not occur with the normal operational predacidal use of sodium cyanide or Compound 1080. After two days' exposure on vegetation and soil, only very small amounts of sodium cyanide were detected [*20*], and Compound 1080 is degraded by microorganisms in the soil [*21*].

To prevent the potential poisoning of scavengers, residue levels in coyotes at the time of death should not be harmful. And with the use of toxic collars there should be no residues or only a minimal tolerance level, so that surviving collared livestock could still be marketed. If collared livestock die after an attack, as is usual with Compound 1080 collars, residues in or on the carcass should not be poisonous to nontarget animals.

Cost of Chemical and Availability

For obvious economic reasons, it is desirable that predacides be inexpensive and readily available from commercial sources. The cost of Compound 1080 is $25 per pound, and about $0.02 worth is needed to fill a toxic collar. In contrast, the experimental chemical PAPP costs $737 per pound, and the amount in one toxic collar costs about $19.50. In a single-dose bait containing 5 mg of Compound 1080, the toxicant cost per bait is only 0.03 cents, but the cost of PAPP in a drop bait is 0.24 cents. Based on these figures, PAPP may be cost-effective for baits but not for toxic collars. The popular carbamate insecticides methomyl and carbofuran may have uses as predacides and are readily available from agricultural supply stores at $13 to $25 per pound.

Chemicals such as PAPP, which do not have established commercial uses, are usually high priced and available in only small quantities. The logistics of getting them into predator control use could be burdensome and because of the political, emotional nature of predator control, especially with toxicants, some chemical companies do not want to be associated with predacidal applications and will not provide their chemicals to be used even for laboratory tests.

Regulatory Affairs

When all efficacy, safety, and environmental hazard criteria appear to be met, it is still necessary to obtain EPA registration for use of any predacide. Before the product can be registered, the applicant may be required to evaluate the chemical under actual field conditions under an EPA experimental use permit. Field tests may require years of expensive work in different geographical regions. Experimental use of Compound 1080 in toxic collars, for example, has been continuous from May 1978 to the present time. As of March 1982, there are three active experimental use permits in the United States. Test results show that the Compound 1080 toxic collar can be used in many situations to stop predation on livestock but that it is not a panacea for all livestock operations [*17*]. The FWS submitted an application to register the Compound 1080 toxic collar in September 1981, and an EPA decision on this application is not expected before late 1982.

We believe that the eight criteria presented in this paper represent the most important factors needed to select, test, and develop predacides for registration and ultimate operational use. These criteria are only broad categories which represent the more than 150 tests that may be required by the FIFRA. The final selection of a predacide is a compromise because no chemical fully meets all the criteria. In reviewing the various criteria to reach a final decision, human and environmental safety receive the highest priority.

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Philip S. Gipson[1]

Evaluation and Control Implications of Behavior of Feral Dogs in Interior Alaska

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ABSTRACT: Feral dogs (*Canis familiaris*) were studied from August 1979 through December 1981 in interior Alaska. Study techniques included use of radio telemetry, tracking in snow, and direct observation. Data were obtained on feral dog life history, including home ranges, activity patterns, breeding activity, foraging behavior, and interactions with tame dogs, red foxes (*Vulpes vulva*), coyotes (*C. latrans*), and wolves (*C. lupus*). Study findings challenged the hypothesis that populations of feral dogs or of dog-coyote or dog-wolf hybrids will not survive in northern regions because they are generally less fit than native canids and have lowered survival of offspring. Adult feral dogs and their pups survived winter conditions and were commonly active when temperatures were between 29 and −30°C. On one occasion, pups and their mother foraged away from the den when temperatures were below −45°C. Interactions of feral dogs with man and damage to livestock and game animals were surveyed. Periodic attempts to reduce feral dog populations have been undertaken by local residents. The principal control measures they employed included shooting, trapping, snaring, and destruction of pups in dens. These control measures resulted in temporary local population suppression. Information determined from this study should be helpful in planning future control measures, if control is deemed appropriate.

KEY WORDS: feral dogs, vertebrate pest control, garbage dumps, canid behavior

Feral dogs exist in many parts of the world where climates are mild [*1-3*]. Mengel [*4*] hypothesized that populations of feral dogs, dog-coyote hybrids, or dog-wolf hybrids cannot withstand harsh winters, especially if coyotes or wolves are present. Dogs give birth year-round, and the breeding cycle of dog hybrids is generally shifted to summer or autumn, so pups are often born in

[1]Assistant unit leader, Alaska Cooperative Wildlife Research Unit, University of Alaska, Fairbanks, Alaska 99701.

winter when survival is unlikely. The absence of care of pups by dogs or dog hybrids other than the mother may prevent establishment of populations of feral dogs or dog hybrids. Also, wolves commonly kill and eat dogs [5]. Young [6] reported accounts of dog-wolf hybrids in northern Canada and Alaska, but there are no recent published reports of feral dogs or dog hybrids. The hypothesis that populations of feral dogs or dog hybrids cannot occur in northern regions is widely accepted.

I received reports of feral dogs in interior Alaska during 1977 and 1978. To determine the validity of these reports 207 questionnaires seeking information about feral and free-ranging dogs were mailed to professional wildlife biologists and hunting guides working in Alaska.[2] Feral dogs were defined as dogs without direct dependence upon man. Twenty-six reports of feral dogs were received.

Game biologists and residents reported that feral dogs had been present near Delta Junction, Alaska, since construction of the Alaska Highway during World War II and possibly earlier. In 1980 Delta Junction had a human population of 945 and covered approximately 3 km^2. The population of feral dogs near Delta Junction was studied from summer 1979 through 1981 to determine the size, distribution, and social organization of the population. Interactions of the feral dogs with man, livestock, and wild animals were investigated. Methods of controlling wild dogs were reviewed.

Methods

Six feral dogs were captured in No. 4, double-spring steel traps with offset jaws and radio-collared during 1979 and 1980. Locations of radio-tagged dogs were determined one or more times each month from November 1979 through December 1981. Locations of radio-tagged dogs were determined with portable receivers and directional antennas carried by hand or mounted on automobiles or aircraft. Whenever practical, an attempt was made to observe radio-located dogs to determine their behavior and whether they were alone or accompanied by other dogs.

Trails of dogs in snow were backtracked from radio locations to determine hunting behavior, favored use areas, and interactions with other animals. Scats found along trails of feral dogs and at favored use areas were collected to determine foods eaten.

Observations were made from blinds or concealed automobiles at dog dens, rendezvous sites, and garbage dumps for 1 to 24 h. From late May through July there is no darkness in interior Alaska, and observations were made continuously for up to 24 h without lights. During other months observations at night were aided with spotlights.

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Carcasses of dogs, coyotes, wolves, and foxes killed near Delta Junction by trappers were collected and examined. Local residents were interviewed to obtain information about feral dogs and other canids. Techniques used by local residents to reduce feral dog populations were noted.

Results and Discussion

Life History of a Pack of Feral Dogs

We first sighted a pack of seven adult feral dogs about noon on 14 Aug. 1979 near a den 250 m east of the Delta Junction garbage dump (old dump in Fig. 1). These dogs were surprised at close range by a field assistant who was moving quietly, upwind, along a game trail. The initial response of the dogs was to bark aggressively and approach the intruder. When the dogs caught his scent, they turned and fled. This response proved to be typical of this pack and on four other occasions we were able to locate the pack by radio signals from one or more dogs and approach close enough to count and identify members of the pack. The dogs had an elkhound/huskylike appearance, except for one black dog that appeared to be mostly German shepherd and one stocky white dog with large black spots. All of the dogs had long, straight hair, similar to adult coyote hair. The members of this pack always appeared to be in excellent physical condition, except for pups born during autumn 1980, which apparently died from exposure when the den was abandoned. This pack was studied through December 1981.

The study pack ranged over an area of approximately 70 km^2 (Fig. 1). Their favored foraging areas were garbage dumps, riparian habitats along the Delta River and brush, and black spruce forests along the Trans-Alaska Oil Pipeline and an abandoned military pipeline. The size of this pack ranged from eleven in August 1980, down to three in the winter of 1980–1981. The maximum size of eleven was reached when four pups, about eleven months old, became fully participating pack members. The low pack size was reached when trappers caught eight adult dogs and all the pups born during autumn 1980 died.

A litter of five pups, three males and two females, was born during late September 1979 at a den 200 m north of the Delta Junction garbage dump (Figs. 1 and 2). Four of these pups survived through the winter of 1979–1980; one pup was killed in March 1980 when struck by an automobile 2 km north of the den. All of these pups were similar in appearance: brown bodies, gray to black tails and leg trim, and dark gray faces with a white blaze on the forehead. A second litter, born to the same female during September or October of 1980, appeared identical to the pups born during 1979. The similarity of the pups suggested they all had the same father.

The mother of the pups was captured and outfitted with a radio collar in November 1979. She visited the den for periods of 15 min to 2 h every one to

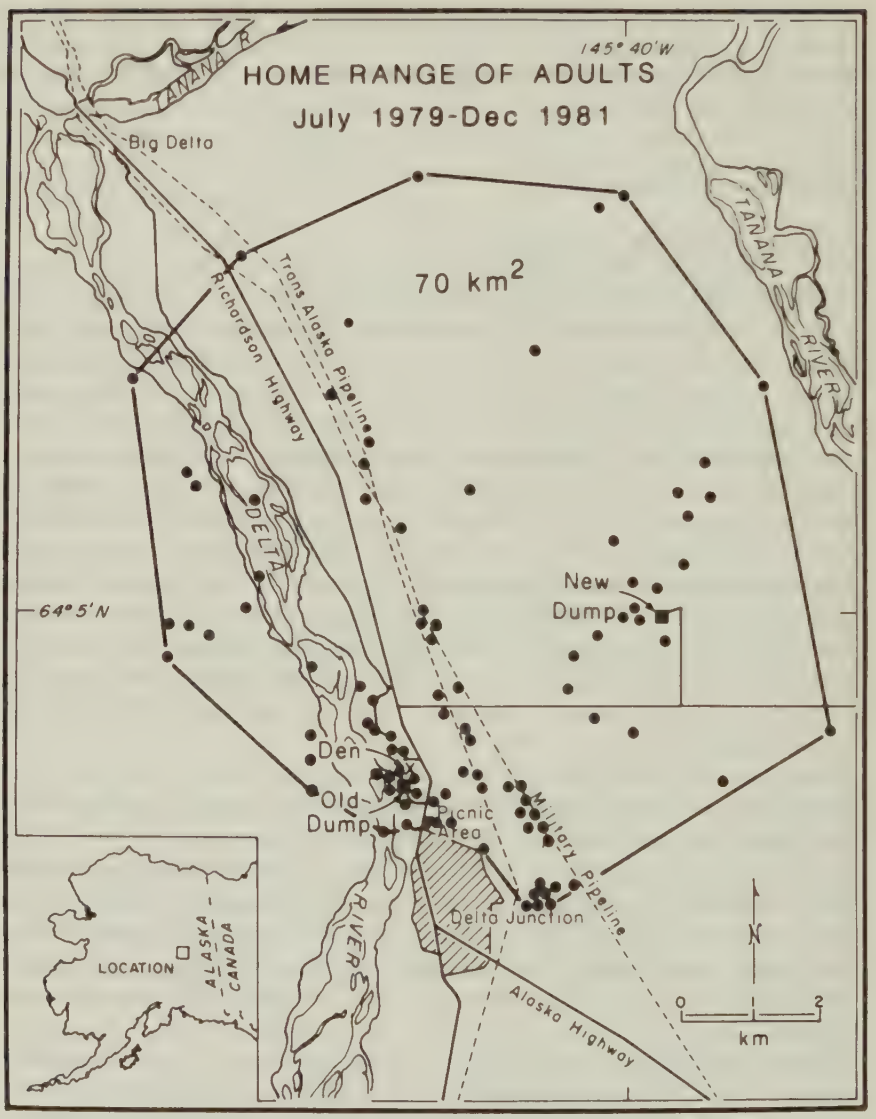

FIG. 1—*Home range of the study pack of feral dogs from July 1979 through December 1981. The dots represent the locations of adult members of the study pack determined by radio triangulation or visual observations, or both.*

three days from November 1979 through February 1980. On at least two occasions a second adult dog accompanied the female to the den, and often tracks of other adults were noted around the den. The pups started to forage independently at the dump in November. They continued to range farther from the den until it was abandoned during March 1980. Cold weather did

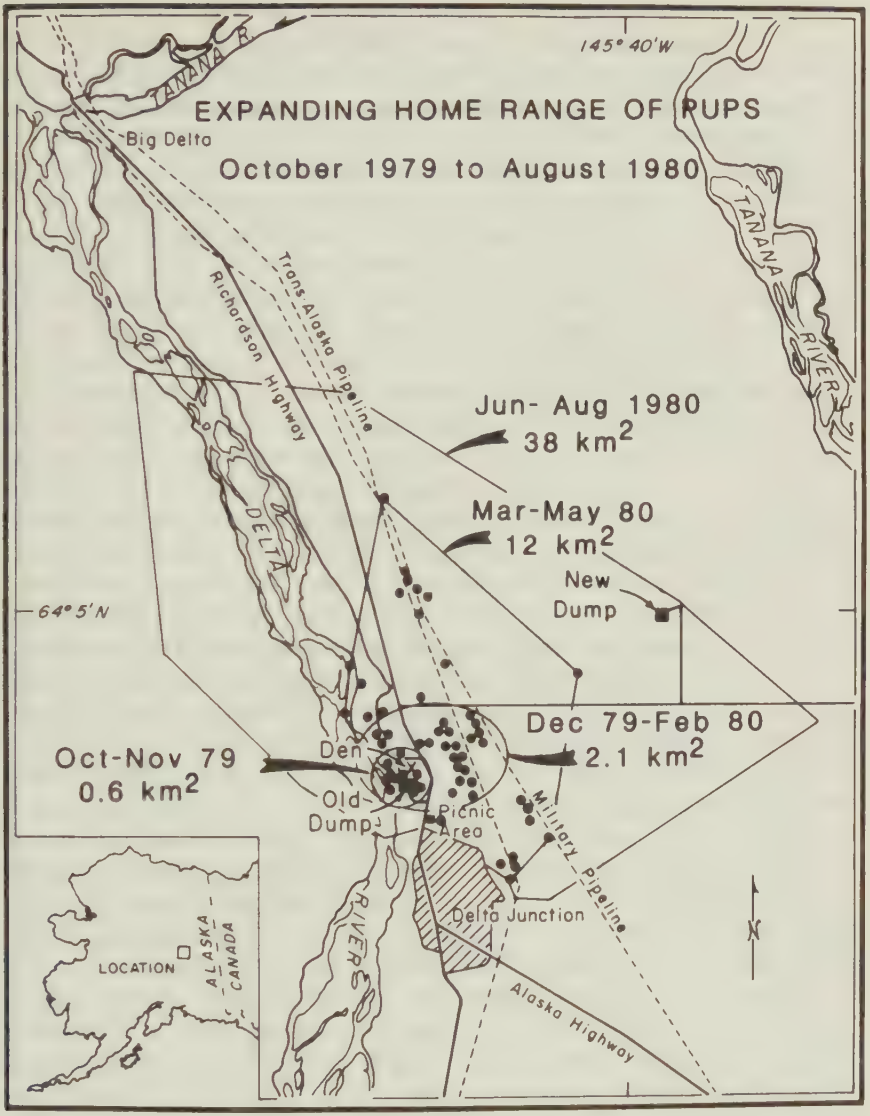

FIG. 2—*Expanding home ranges of a litter of feral dog pups from soon after birth in autumn 1979 until they were fully integrated into the pack in August 1980. The dots represent the locations of the pups determined by radio triangulation or visual observations, or both.*

not seem to affect the dogs. Pups and adults were commonly active when temperatures were between 29 and −30°C. Once in December 1980, the mother and five pups foraged away from the den when the temperature was −45°C and winds were 6 to 8 km/h.

The pups gradually increased the size of their home range (Fig. 2) and their

socializations with the pack. By August 1980 the pups were traveling regularly with the pack over most of the pack's home range. During mid-December 1979 the pups were first observed loafing with the pack in a wooded area near the den. After that, the pups were often seen or located by radio signals on forays with their mother or a large black and white male dog believed to be their father, or with both adults. An area along the Trans-Alaska Oil Pipeline, about 4 km northeast of the den, was a favored resting area for the pups, their mother, and the black and white male after February 1980.

During summer 1980 the pack shifted its primary use area from near the old garbage dump approximately 5 km northwest to the new dump (Fig. 1). A den was established during summer 1980 in a large pile of stumps near the new dump. These stumps were hauled to the area from recently cleared agricultural fields. The radio-collared female gave birth at this den during late September or early October 1980. The stump pile was then discovered by residents of Delta Junction seeking fire wood. The female and other dogs fled the den on at least two occasions as people approached to cut fire wood. Harassment from gatherers of fire wood apparently caused the dogs to abandon their den during late October and transfer the pups to a rendezvous site 1 km west of the new dump. By that time the ground was frozen, and digging a new den would have been extremely difficult. By mid-November 1980 there was 20 to 30 cm of snow on the ground, and temperatures dropped from 0 to −5°C at night. Apparently several pups died from exposure during November. Only one pup was still alive when the pack was sighted at the new dump on 25 Nov. 1980, and this pup was leaving drops of blood in its tracks.

During the first week of December 1980 temperatures dropped to −40°C. When the study team located the pack on 7 December no fresh sign of pups could be located, and beds formerly used by pups at the rendezvous site were covered with snow. The last pup apparently died during the extremely cold weather of December 1980. The presence of dens to protect pups from severe weather and a dependable food source such as garbage dumps seem to be required for dogs to successfully rear young in interior Alaska.

Sightings of tracks and reports from trappers suggested that at least one additional pack of feral dogs existed along the Tanana River, north of the intensely studied pack. Three individual feral dogs and two pairs that appeared to be independent of the packs were also sighted in the area. The total population of feral dogs around Delta Junction was estimated at 17 to 30.

Interactions with Other Canids

Feral dogs and free-ranging tame dogs remained segregated most of the time. Numerous tame dogs ranged freely from houses in the area. Photos were made of known free-ranging dogs, and the locations of their homes were noted. This aided in determining movement patterns of unconfined tame

dogs in relation to feral dogs. Free-ranging tame dogs tended to travel along roads and to stay near houses. Only one tame dog regularly visited the new garbage dump, and we did not see this dog interact with the feral pack. A local hunter, Frank Creamer, told of calling in a group of wild dogs with a predator call, and one of the dogs was wearing a collar. Creamer later caught this dog in a snare, and he indicated that the dog was aggressive and showed no sign of being tame when he approached. This dog may have become wild and joined the pack. There were no other indications that members of the feral pack were recruited from abandoned or lost dogs.

Feral dogs often interacted with coyotes, red foxes, and occasionally wolves. Coyotes and foxes are common in the Delta Junction area, and during the winters of 1979–1980 and 1980–1981 a pack of three wolves passed through the area about every seven to ten days. The dogs commonly used trails traveled by coyotes and foxes. Dogs urinated on scent posts established by coyotes whenever they were encountered, however, scent stations established by foxes were usually ignored.

Scent stations established by wolves and even the trails of wolves elicited a great deal of interest among feral dogs. On three occasions the study pack scratched in the snow and urinated numerous times around spots where wolves had urinated. Sign in the snow indicated that some of the dogs rolled on the wolf urine. Twice trails of the feral dog pack on gravel bars along the Delta River turned abruptly into the forest, away from trails of wolves they encountered. Only one report of a dog associating with a wolf was received. Edward Sheehan, a well-known trapper in the area, told of seeing a dog running with a wolf during the winter of 1969–1970 along the Delta River upstream from Delta Junction. He described the dog as about as tall as the wolf, but more massive, with a dark brown body and black tail and shoulders. According to Robert Stephenson, wolf biologist for the Alaska Department of Fish and Game, more than 30 dogs were killed and some eaten by wolves on the outskirts of Fairbanks during the winter of 1974–1975. Possibly feral dogs near Delta Junction have been able to survive in the presence of wolves by avoiding them and by running in packs.

Foraging Behavior

The diet of feral dogs was varied, but there was a strong dependence on garbage and small animals (Fig. 3). Scraps of meat and bones were favored foods at the dumps. Dogs observed feeding at garbage dumps were highly tolerant of each other. The entire pack or groups of two to four dogs would usually enter the dump together, and occasionally dogs approached each other and fed side by side from the same garbage pile.

Sign in the snow indicated that dogs often spread out over brushy areas to hunt for snowshoe hares (*Lepus americanus*). The trails of all the dogs in-

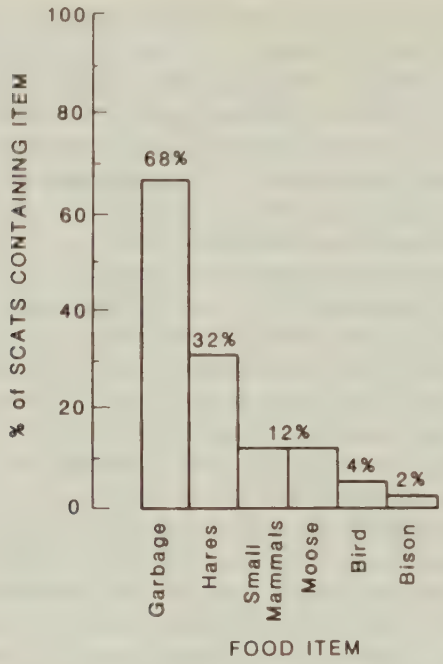

FIG. 3—*Remains of food items identified in a preliminary analysis of 42 scats from feral dogs collected during the winter of 1979–1980.*

volved in a chase converged on the capture site when a hare was caught. The hare was apparently eaten immediately. Only a few drops of blood, hairs, and occasionally the feet of hares were found at catch sites.

Damage Reports

Three reports were received of dogs chasing cattle, but no kills were reported. We were not able to determine if the cattle were harassed by feral dogs or by free-ranging dogs. Several sheep were killed by predators near Delta Junction in 1978, and the owner felt feral dogs were probably involved. One report of feral dogs attacking a yearling moose (*Alces alces*) was received. The observer described six to ten dogs, including a "large black and white dog and several smaller gray husky crosses," that fit the appearance of dogs in the study pack. The moose was attacked in a favored foraging area for the dogs. One of the moose's ears was partly torn away, and there was bleeding from cuts on the nose and hips. The dogs and the moose fled when the observer called out. Remains of moose and bison (*Bison bison*) found in dog scats could have come from kills made by the dogs, from moose and bison parts discarded by hunters, or from carcasses of animals dying of other causes.

Two residents of Delta Junction reported losses of pet male dogs that they thought were killed by feral dogs. The carcass of one of the dogs was discovered near a number of junked car bodies at a favored loafing area of the feral dog pack. The tame dog had clearly been killed and torn apart by canids. The second dog was never found. In a third case a female cock-a-poo was attacked by several dogs while she was in heat. A large dog was reportedly dragging the cock-a-poo when the owner frightened the dogs away. Two other instances were reported of strange dogs, believed by the observers to be wild, approaching tame female dogs that were in heat. One individual was bitten by an apparent wild male dog when he approached the male and the tame bitch while they were copulating.

There were no reports of feral dogs attacking people, other than the aforementioned case where a man was bitten. Four people did report surprising groups of feral dogs when the dogs barked and approached until they saw or scented the people. In each case the dogs fled when they were aware that people were near. One case was reported in which a possible feral dog darted from the woods and stole a bag lunch when children were playing several metres away while waiting for a school bus.

Control Efforts

Several times during the past ten years residents of Delta Junction have organized informal efforts to reduce or eradicate the feral dog population. Charles Champlaign and several companions reported killing approximately 60 dogs during 1976 and 1977. Their approach was to post gunners in blinds near a den complex containing dog pups, approximately 4 km northeast of the old garbage dump, while a second team of gunners waited in hiding at the dump. When feral dogs entered the dump to feed, the gunners shot as many as possible. Many of those that escaped from the dump fled to the den and several more dogs were killed there by the waiting gunners.

Rodney Walters and John Buris initiated an informal control effort during 1974 when feral dogs had a den in an abandoned hunting lodge south of the Alaska Highway. These men established blinds near the den and waited for dogs to visit the den. Approximately 30 dogs were reportedly killed near this den.

Frank Creamer told of catching six apparent feral dogs in snares during the winter of 1977–1978. He snared four more dogs in autumn 1979, and the same autumn he called in several more with a predator call and shot two of them.

Other sporadic attempts to reduce the population have concentrated on trapping, snaring, shooting dogs at the garbage dump, and finding dens and destroying pups. The numbers of feral dogs reportedly killed between 1974 and 1978 exceed our estimate of the total population (17 to 30 dogs) during 1979, 1980, and 1981. The Trans-Alaska Oil Pipeline was under construction from 1974 through 1977, and it is possible that the population of feral dogs increased

markedly during that period in response to additional food available at dumps near work camps and garbage discarded at construction sites.

Results of the present study suggest that when control is deemed advisable, feral dog populations should be relatively easy to control using techniques developed for controlling coyotes [7]. Den hunting, shooting, and trapping are effective and selective. Use of toxicants is usually not appropriate since feral dogs tend to be present in settled areas where people, pets, and prized furbearers are common. Closing or fencing open garbage dumps would aid in reducing feral dog populations.

Summary

Feral dogs have existed near the community of Delta Junction, Alaska, for 30 years or longer. These dogs interact socially with coyotes, wolves, and tame dogs in the area. Red foxes and their scent stations are often ignored by the dogs. The feral dog population was estimated at 17 to 30 during 1979, 1980, and 1981. Reports from game biologists and local trappers suggest the feral dog population may have been considerably higher during construction of the Trans-Alaska Oil Pipeline from 1974 through 1977. The only known recruitment by the pack was through reproduction among members of the pack and integration of pups into the pack.

The principal foods of feral dogs were garbage, snowshoe hares, and small mammals. Moose and bison tissue was eaten, but it is not known if the dogs killed moose and bison or if they fed on remains of sport hunter kills. Wild birds made up a small, but consistent, part of the diet of feral dogs.

When control of feral dogs is deemed appropriate, shooting, destruction of pups at dens, trapping, and snaring can be effective. Elimination of open garbage dumps, or possibly fencing such dumps, would aid in reducing feral dog populations.

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Summary

Summary

In this fourth volume in a series of symposia proceedings dating from 1976 and dealing with vertebrate pest control and management materials (see also *ASTM Special Technical Publications 625, 680,* and *752*), the ubiquitous pest rodent (14 papers), bird (8 papers), and canine (3 papers) species that have been well-represented in prior volumes are again the study of researchers in government, university, extension programs, and private industry. This continued attention to these animals reveals the divergent aesthetic, economic, and public or animal health problems the species continue to cause mankind. They also reflect the sustained research needs of seeking better understanding and management of these animal problems.

The research methods described in this volume offer further means toward that understanding and management. Basic verification of the value of traditional management and control materials has been the subject of much prior work with the major pest species such as the commensal rat. With these species, the present volume contains several papers that extend and improve prior laboratory and field methodology or devise newer ones to optimize familiar control techniques, such as improved efficacy, lower cost, or less hazard. Other papers extend prior methods to evaluations under additional special circumstances. Still other authors seek to reduce variability and improve the significance, reliability, and predictive value of the data obtained.

Accordingly, in bird research *Bullard et al*, *Tobin and DeHaven*, *Heisterberg and Otis*, and *Shumake et al* offer new designs in laboratory treatment or exposure regimens for toxicants or repellents, or present methods to enhance or optimize available materials or to overcome limitations of earlier methods. In rodent research, *Stewart et al* extend psychophysical techniques to the characterization and improvement of rodenticides.

As yet, such an advanced research state has not been equally reached with all species and all control and management aspects. A key review by *Reidinger and Mason* is a first attempt at a comprehensive synthesis and summary of the diverse arenas of aversive behavior research in rodents and birds and overcomes the dichotomy often apparent between animal psychologists and applied biologists. In another important review paper, *Schmidt and Johnson* are concerned with bird dispersal recordings, an area that has suffered from a sparsely documented and seldom researched background. From these beginnings, others can more easily proceed to the design of meaningful evaluations

to quantify pest animal responses, compare available management options, and devise optimum control strategies.

Such preliminary steps in creating laboratory research evaluation methodologies are illustrated in this volume with an examination of an old, yet largely unresearched, control technique of glueboard traps. These devices have been recently resurrected in view of the decline of available toxicant materials effective on mice. Methods for the study of the efficacy of glueboards against house mice are presented by *Frantz and Padula*. A new control approach involves the development of a rodenticide applicator that applies a toxic liquid to the fur of mice that enter the device and subsequently ingest the toxicant by grooming. The necessary testing methodologies in the development of such a device are presented by *Morris et al*. A similarly broad development program to produce an effective coyote lure is described by *Fagre et al*. from initial laboratory characterizations to field response determinations.

More specific contributions, but still essential keys to allow further research, are represented in methodologies that standardize, quantify, or adjust variance in such testing aspects as the materials that suffer vertebrate pest damage or variability in the access by pest species to such materials. Examples in bird research involve grape attack in the paper by *Tobin and DeHaven* and damage to rice sprouts in the paper by *Otis et al*.

Generally, it is only with a clear understanding of the prior art of vertebrate pest control and use of management materials and a thorough understanding of the animals themselves in the field and the problems they create that effective methods and materials can be best studied and developed under controlled conditions. Yet, traditional techniques in field biology for the study of animals and animal populations are frequently limiting in the type of data that can be obtained. For example, basic, defensible techniques for abundance and damage determinations are lacking for many species and situations. *Fagerstone* makes a valuable contribution in illustrating methods to validate visual observations of ground squirrels as a census technique. *Gipson* adds the potential for determining behavioral components in studies of feral dogs in the unusual conditions of the Arctic. *Anthony and Barnes* compare abundance techniques for gophers and the correlation of such techniques with observed conifer damage. Similarly, *Sultana et al* are concerned with assessing wheat damage by rodents in Bangladesh.

While field observations are invaluable, many vertebrate pests, especially rodents, are secretive and may instead require artificial conditions before key behavioral aspects can be discerned. *Elias et al* describe an artificial burrow system for wild rodents, and *Glahn and LaVoie* present an apparatus to record rodent attack on food container materials visually.

Work with wild species in confined conditions is difficult because of stress and sources of potential variation in animal health or response to captivity. Yet, sufficient normal behavior to allow for meaningful determinations, such as in taste preferences, must be retained. Correspondingly, to preserve this es-

sential normal behavior, techniques of immobilization, surgical procedures, or unavoidable negative stimuli are *not* typical components of animal preparation or testing methodologies in vertebrate pest research. Accordingly, and because of the recognized pest status of the animals involved, only limited attention has been focused on such research by those raising the questions of animal rights and animal protection in the scientific community. Nevertheless, humane aspects of testing, management, and control alternatives should be important considerations in research with vertebrate pests and in the application of this research to the field. Although the call for papers for this symposium included a suggested topic relating to humane considerations in vertebrate pest management and research, no papers were received on this subject. Perhaps it will be useful to address this area more specifically in subsequent symposia.

In the present volume, special attention is given to techniques for working with pest bird species in confinement by *Bullard et al*, *Tobin and DeHaven*, *Heisterberg and Otis*, and *Merson et al*. The authors optimize confining, feeding, handling, maintenance, and testing conditions to allow meaningful test results to be obtained for the species and in the specific test situations with which they are concerned. Such recommendations and practices cannot arise from the "armchair scientist." Rather, they are laboriously acquired from the observations and intuition from extensive familiarity with the particular species involved, if not painfully acquired by failures in prior experimentation.

Methodologies, however sophisticated, for laboratory evaluations of captive pest vertebrates may have little application to the needs of field investigations and vice versa. The need for research information that bridges the abrupt "lab-field" testing transition can often be met by seminaturalistic test designs that allow pest species under test some greater semblance of natural environmental conditions and provide for social interaction while reducing the variables, uncontrolled effects, and often extensive resource needs of actual field evaluation techniques. *Ashton, McCumber, and Jackson* propose such a test design for rodenticide evaluations with small rodents in an outdoor enclosure, and *Morris et al* describe an indoor simulated warehouse enclosure for house mice. A proposed technique by *Merson et al* allows bird hazard from ingestion of particulate broadcast pesticides to be evaluated under outdoor conditions yet in a replicated, semicontrolled fashion. One aspect of the paper by *Bullard et al*, as well, includes a field enclosure technique to make possible testing of birds under more natural conditions.

Ultimately, vertebrate pest control and management techniques require field verification as candidate methods and materials emerge from laboratory and seminaturalistic testing paradigms or are suggested from observations of natural behavior of pest animals. While few new wrinkles on existing evaluation techniques (such as relative census methods) are presented in this volume, the emphasis is on relative comparisons of the suitability and consistency of these existing census methods in field situations. Examples for commensal

rodents are the papers by *Spaulding and Jackson* and by *Mathur and Prakash*; for coyotes, the paper by *Fagre et al. Ashton, Jackson, and McCumber* argue that existing methodologies for censusing commensal rodents and careful site selection can allow comparisons to be made of different control methods or materials (or comparison with untreated sites) in the same or in comparable evaluations. This can allow selection of optimum control methods and materials for a particular situation, or allow investigation of the components of the integrated pest management (IPM) programs being so strongly proposed today. The paper by *Spaulding and Jackson*, as well, illustrates how computer technology can aid in field data recording and in subsequent analysis of commensal rodenticide field evaluations.

Many papers list the optimum characteristics of a specific management or control material, such as the paper by *Fagre et al* on coyote lures. Such characterizations by those intimately involved in the evaluation of such techniques are valuable to all. The results of research in both controlled and natural conditions with vertebrate pests ultimately shape the application of these findings to the actual management and control of these harmful vertebrate species. Thus, the decision-making process by regulatory authorities, control specialists, and manufacturers of control methods or materials must properly involve a good balance of relevant vertebrate pest research input, in combination with information on economic damage thresholds or health hazards and the economics and public acceptance of particular management or control options. The factors involved in such decision-making processes are well-illustrated in the papers by *Salmon and Lickliter* and by *Savarie and Connolly*. The considerations such papers illustrate provide valuable guidance in ensuring that optimum control and management choices can be made. Previous inadequate information, simplifications, and errors by regulatory groups, manufacturers, and others, for example, have contributed to widespread misconceptions about management and control material needs, actual product or method field performance, and nontarget hazard in specific field conditions. A rapidly dwindling number of alternative registered materials for vertebrate pest control has resulted. The United States and many other countries appear already to have less than the "critical mass" of available alternative control and management materials necessary to allow the best *combination* of such approaches in integrated programs. Such integrated approaches can often most effectively reduce the potential for problems developing with the overuse of a particular tool (for example, toxicant resistance or method aversion) and can best allow necessary control levels to be achieved in specific vertebrate problem situations at minimum risk, cost, and delay.

In conclusion, the collective papers of this volume reflect attention to a considerable extent of different pest species, pest problems, and research approaches—from blackbirds to bandicoots, precise laboratory paradigms to inner city slum "plots," Arctic to desert to tropic situations, and from the isolation and study of specific testing components to descriptions of the entire

"from lab to field" process of development of a control or management option. Evaluation methods for toxic control agents, particularly for rodents, are well-represented, as are repellent evaluations for birds.

Those looking for specific data will find a limited amount in these papers, for example, on repellents like methiocarb or anticoagulant rodenticides. Generally, however, such actual data have been included only to illustrate and verify the methods that generated the data. This practice is consistent with the goals and style requirements of ASTM in encouraging the development and dissemination of testing methodologies. Readers interested in specific data will find useful the proceedings of the various Vertebrate Pest Conferences, which have been held in conjunction with these ASTM symposia, as well as predating the conception of these symposia in volumes extending back to 1962.

It is notable that there are apparently few examples in these ASTM symposia proceedings of general test methodologies first being developed and *then* a specific treatment or response variable being fitted to the method. Rather, in the truest sense of the process of applied science (and probably due to the trend of reduced funding to university and government research facilities), nearly all the authors have been attempting to answer specific research needs with specific species and control or management materials when they devise their particular test procedures. Fortunately for their colleagues, their diligent efforts to succeed in their specific cases may well have application in other areas. As an example, the test designs adopted by *Heisterberg and Otis* with pest birds were originally utilized in livestock feed research.

These volumes offer a wealth of leads and ideas, as well as readily transferable techniques, to solve research needs with yet other species, situations, and management approaches. The findings, problems, limitations, and advice for improvement represented by the many authors' papers reflect the difficulty of their work. Also reflected are the honesty and dedication of the authors in their search for ever-improved techniques to allow the alleviation of the vertebrate pest problems that so seriously affect the health and well-being of so much of mankind. Correspondingly, this volume represents the tremendous value of ASTM in providing an outstanding vehicle for dissemination of information on the concerted efforts of the scientific community in this valuable area of research.

Dale E. Kaukeinen

ICI Americas Inc., Biological Research Center, Goldsboro, N.C. 27530; symposium chairman and editor.

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